Vol. 31, No. 2 June 1954

Illinois U Library

The Journal of
Experimental Biology

EDITED BY
J. GRAY and J. A. RAMSAY

Contents

PAGE

Anne Rowlands. The influence of water and light and the pituitary upon the pigmentary system of the common toad (*Bufo bufo bufo*) 151

Kathleen M. Downing. The influence of dissolved oxygen concentration on the toxicity of potassium cyanide to rainbow trout 161

Barbara H. Dainton. The activity of slugs. I. The induction of activity by changing temperatures 165

Barbara H. Dainton. The activity of slugs. II. The effect of light and air currents . . 188

W. A. Mannell and R. J. Rossiter. Wallerian degeneration in the rat. The effect of age on the concentration of nucleic acid and phospholipid in intact and sectioned nerves 198

Martynas Yčas. The respiratory and glycolytic enzymes of sea-urchin eggs . . . 208

D. A. Parry. On the drinking of soil capillary water by spiders 218

H. J. Thomas. The oxygen uptake of the lobster (*Homarus vulgaris* Edw.) . . . 228

Lord Rothschild and A. Tyler. The physiology of sea-urchin spermatozoa. Action of versene 252

G. Hoyle. Changes in the blood potassium concentration of the African migratory locust (*Locusta migratoria migratorioides* R. and F.) during food deprivation, and the effect on neuromuscular activity 260

Peggy E. Ellis and G. Hoyle. A physiological interpretation of the marching of hoppers of the African migratory locust (*Locusta migratoria migratorioides* R. and F.) . . 271

V. J. Wilson. Slow and fast responses in cockroach leg muscle 280

E. R. Trueman. Observations on the mechanism of the opening of the valves of a burrowing lamellibranch, *Mya arenaria* 291

Published for The Company of Biologists Limited
CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON

AGENT FOR U.S.A.
ACADEMIC PRESS INC.,
125, EAST 23RD STREET, NEW YORK, U.S.A.

Price 30s. net *Subscription per volume 84s. net*

THE JOURNAL OF PHYSIOLOGY

MAY 1954. VOL. 124, NO. 2

WADE, O. L. Movements of the thoracic cage and diaphragm in respiration.

BRADLEY, R. D., GASKELL, P., HOLLAND, W. W., LEE, G. DE J. and YOUNG, I. MAUREEN. The acid-base changes in arterial blood during adrenaline hyperpnoea in man.

FELDBERG, W. and SMITH, A. N. The role of histamine release for the motor effects of histamine liberators on the guinea-pig's ilium preparation.

HUTTER, O. F. and KOSTIAL, KRISTA. Effect of magnesium and calcium ions on the release of acetylcholine.

HARRIS, E. J. An effect of stretch upon the sodium output from frog muscle.

HARRIS, E. J. Ionophoresis along frog muscle.

CRAGG, B. G. The electrical responses of mammalian cerebral cortex.

PRINGLE, J. W. S. The mechanism of the myogenic rhythm of certain insect striated muscles.

MACPHERSON, L. and WILKIE, D. R. The duration of the active state in a muscle twitch.

HUMPHREY, J. H. and TOH, C. C. Absorption of serotonin (5-hydroxytryptamine) and histamine by dog platelets.

HUMPHREY, J. H. and JAQUES, R. The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species.

MERTON, P. A. Interaction between muscle fibres in a twitch.

DOUGLAS, W. W. and PATON, W. D. M. The mechanisms of motor end-plate depolarization due to a cholinesterase inhibiting drug.

ALLWOOD, M. J. and BURRY, H. S. The effect of local temperature on blood flow in the human foot.

CORT, J. H. and MCCANCE, R. A. The renal response of puppies to an acidosis.

DEL CASTILLO, J. and ENGBAEK, LISE. The nature of the neuromuscular block produced by magnesium.

LANCE, J. W. and MANNING, R. L. Origin of the pyramidal tract in the cat.

BRINDLEY, G. S. The summation areas of human colour-receptive mechanisms at increment threshold.

BOURNE, GEOFFREY H. The histochemical dephosphorylation of oestrogen phosphates.

Subscription price 70s. net per volume of 3 parts

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1

The Journal of
Experimental
Biology

INDEX
to
VOLS. 1–15

15s. net

To members of the Society
of Experimental Biology, 7s. 6d.

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD
LONDON, N.W.1

SYMPOSIA OF THE
SOCIETY FOR
GENERAL MICROBIOLOGY

Autotrophic
Micro-organisms

EDITED BY
B. A. FRY & J. L. PEEL

Thirteen papers given at the fourth Symposium of the Society for General Microbiology held in April 1954. 25*s. net*

CAMBRIDGE
UNIVERSITY PRESS

VOLUME 31, NO. 2 JUNE 1954

THE INFLUENCE OF WATER AND LIGHT AND THE PITUITARY UPON THE PIGMENTARY SYSTEM OF THE COMMON TOAD (*BUFO BUFO BUFO*)

BY ANNE ROWLANDS, PH.D.
Department of Biology, Northern Polytechnic, Holloway, London

(*Received* 6 *December* 1952)

INTRODUCTION

It has been shown (Rowlands, 1950) that water has a significant influence upon the colour change of the frog, *Rana temporaria*: when frogs are immersed in water even upon a white illuminated background, the expansion of the melanophores with resultant darkening is induced.

On the other hand, Parker & Scatterty (1937) see no reason to assume that with the spotted frog, *Rana pipiens*, 'differences in humidity (jars dry—jars 10 cm. water) played any part whatsoever in the final responses to the environment'.

Jørgensen (1950) found that in *Bufo bufo*, the injection of $\frac{1}{200}$ to $\frac{1}{2000}$ of either frog or toad pituitary causes the active uptake of water through the skin, whereas in *Rana temporaria* one-twentieth of a gland must be used to elicit such an effect. This fact suggests that the skin of the terrestrial toad is more sensitive than that of the more aquatic frog to the post-pituitary principle related to water balance.

The main purpose of this investigation was to discover the part played by water in the pigmentary effector system of the toad, and to compare the toad with the frog in this respect. Opportunity was also taken to repeat upon the toad some of the earlier work done on the frog and to study the mechanism of the secondary response by means of operations designed to interrupt the paths between the eye and the pituitary.

MATERIAL AND METHODS

Two groups of toads (*Bufo bufo bufo*) were used; one collected locally from a single pond towards the end of the breeding season (25 April 1951); the other obtained from Cornwall in the autumn (16 October 1951). They were fed weekly on lean horse meat and appeared to be maintained in a healthy condition, tending to increase slightly in weight. In between experiments they were kept in small groups (five to six) so that individual recognition by their patterning was possible throughout the investigations. They were kept in green enamel bins under very shady moist conditions.

The methods and apparatus used were identical with those previously described (Rowlands, 1952), with the exception of the illumination—the wattage of the bulbs being reduced from 60 to 25 except where specifically stated. Although there is virtually no web in the toad, it was possible to observe the melanophores under the low power of the microscope in the skin between the digits, and the melanophore index system of Hogben (1930) could be applied to record their degree of expansion.

(*a*) *Blinding*

One of two techniques was used to blind the toads. They were anaesthetized by the injection of 1 c.c. of 15% urethane into the dorsal lymph space; then, either (1) the optic nerve was severed as described previously for the frog (Rowlands, 1952), or (2) the eyes were removed. To remove the eyes, an insertion was made below the upper orbital ridge and the eye pulled forward so that the muscles and optic nerve could be severed and the eye removed from the socket.

(*b*) *Hypophysectomy*

This operation was carried out on the lines described by Hogben (1923). After anaesthetization, a median longitudinal incision was made in the mucuous membrane of the roof of the mouth and a small hole was bored in the parasphenoid at the level of the cross-piece to expose the anterior pituitary. This was removed by a pipette attached to a suction pump. The posterior lobe was loosened by a probe and then removed by suction. The edges of the mucuous membrane were drawn together and the mouth closed.

(*c*) *Cauterization of the infundibular stalk*

Instead of the hole being in the centre of the cross-piece it was bored further forward between the pituitary and optic chiasma. A red hot electric cautery needle was then applied to the infundibular stalk.

(*d*) *Cauterization of the infundibular stalk with hypophysectomy*

This procedure was a combination of the two operations outlined above. Two groups were thus treated. In the one, both the cauterization of the infundibular stalk and the hypophysectomy were performed together, whilst in the other there was a period of weeks between the cauterization and the hypophysectomy.

OBSERVATIONS

(*a*) *Normal chromatic responses*

Throughout the investigations the variations in response was very considerable. Thus in the resting condition (shady, moist $\simeq$ 16 hr.) there was extreme variation between individuals and in the responses of the same individual. This variation in response was not apparently connected with the initial state of the melanophores (i.e. whether the previous day they had been subjected to a paling or darkening environment, or to any temperature changes which occurred overnight ($\ngtr$ 4 C°.). Over a period of 8 weeks the range of melanophore index noted for shady moist conditions is given in Table 1 for a random sample of males and females.

From the examples shown in Table 2 we may conclude that a white illuminated background induces the contraction of the dermal melanophores whilst a black illuminated background causes their expansion. Further, the addition of water sufficient to cause partial or complete immersion of toads upon a white illuminated background is without significant effect. From Table 3 it is seen that the time taken

Table 1. *Variation in μ. (shady moist conditions $\nless$ 16 hr.) over period 8 weeks*

♂'s. 1	$\mu=1+$ to 5	Commonest $\mu=3$ to 5
2	$\mu=1+$ to 5	Commonest $\mu=2$ to 3
3	$\mu=2$ to 5	Commonest $\mu=3$ to 5
4	$\mu=1$ to 5	Commonest $\mu=3$ to 5
5	$\mu=1·5$ to 5	Commonest $\mu=1·5$ to 3
♀'s. 1	$\mu=1+$ to 4	Commonest $\mu=2$ to 3
2	$\mu=1+$ to 4·5	Commonest $\mu=2$
3	$\mu=1+$ to 5	Commonest $\mu=2$ to 3
4	$\mu=1·5$ to 5	Commonest $\mu=2$ to 3
5	$\mu=2·5$ to 5	Commonest $\mu=3$ to 5

Table 2. *October: temperature* 15–19° C.

	Shady moist (≏18 hr.)	White illum. dry		White illum. ½ im. 4 hr.	Shady moist (≏18 hr.)	White illum. dry 4 hr.	White illum. im. 4 hr.	Shady moist (≏18 hr.)	Black illum. dry		Shady moist (≏18 hr.)	Black illum. ½ im.	
		2 hr.	4 hr.						2 hr.	6 hr.		2 hr.	6 hr.
♀'s.													
$\mu1$	2	2	1+	1·5	1+	1+	1	1·5	3−	5	2	5	5
$\mu2$	2+	1+	2	2	1+	1	1	3−	5	5	2+	5	5
$\mu3$	3−	2	2	2+	2	1+	1	4+	5	3·5	1·5	5	4·5
$\mu4$	3−	2	2	1	3	1·5	1+	5	5	5	3−	5	5
$\mu5$	5	1·5	2−	1·5	2	1	1	2	3	5	1+	5	5
$\mu6$	2·5	1	1	1	2	1+	1+	1·5	3	5	2·5	3	5
$\mu7$	5	2−	1	1	3·5	1·5	1+	2·5	5	5	5	5	5
$\mu8$	2−	1+	1+	1+	1+	1	1+	2+	2	2	2	1+	1+
$\mu9$	3−	2−	1+	1·5	2	1·5	2+	3−	5−	5	4+	5	5
$\mu10$	4·5	2−	1+	1·5	3+	1+	1+	5−	5	5	5−	5	5−
♂'s.													
$\mu1$	4	2−	2+	4	3·5	2	4+	3+	5	5	4+	5	5
$\mu2$	2+	1	1	1	2+	1+	1	1	2	3+	1+	5	5
$\mu3$	2·5	1	1	1	1	1	1·5	1·5	5	3	1	4+	5
$\mu4$	1·5	1+	1+	1	2	1+	1+	1	5	5	1+	5−	5
$\mu5$	4+	2	1+	2	2+	2+	1+	4·5	5	5	2+	4	5
$\mu6$	4·5	5	4	3	5	4−	3+	5	5	5	4·5	5	4·5
$\mu7$	2	2·5	2−	1·5	1+	1·5	1+	2−	5	3·5	1	5−	5−
$\mu8$	3·5	1+	1+	1+	4	1	1	3·5	5	5	5	5	5
$\mu9$	5	2	1·5	1+	2	1	1+	2	5	5	2	5	5
$\mu10$	3+	1	1	1	3	1	1	2·5	4	2·5	2	5	5

for the melanophores to reach equilibrium with either a white or a black background is roughly 1 or 2 hr.

The complete absence of light results in extreme variability of the melanophore index, but there are marked indications of a diurnal rhythm with a tendency to contract in the evening.

From Table 4 it would appear that the different levels of intensity of illumination used had little effect upon the melanophores.

One interesting phenomenon repeatedly observed was the tendency of specimens irrespective of the environment to darken several hours prior to moulting and remain dark until the moult had occurred.

The normal chromatic responses can be summarized as below, showing clearly that water elicits no significant melanophore response:

White illuminated dry: $\mu=1$ to 2.
White illuminated half-immersed: $\mu=1$ to 2.
Black illuminated dry: $\mu=4$ to 5.
Black illuminated half-immersed: $\mu=4$ to 5.
$(\mu=4 \text{ to } 5)\rightarrow(\mu=1 \text{ to } 2)=1$ or 2 hr.
$(\mu=1 \text{ to } 2)\rightarrow(\mu=4 \text{ to } 5)=1$ to 2 hr.

(*b*) *Chromatic responses in blinded toads*

All specimens prior to operation showed the normal responses to the various combinations of experimental conditions. Following either the severance of the optic nerve or the removal of the eyes, the reactions of the melanophores to varying environmental conditions were again repeatedly tested over a period of 6 months. Table 5 contains a sample of the results obtained when the optic nerve was severed and Table 6 when the eyes had been removed. In the former group (24 specimens, 12♀'s + 12♂'s) subsequent dissection showed that the optic nerve had been severed and had regressed slightly within the brain case. In the latter group (20 specimens, 10♀'s + 10♂'s) long before the end of the 6-month period the eye sockets were overgrown by the skin from the upper and lower ridges meeting. The two operations as was expected gave essentially the same results. There was in all specimens an apparent loss of all co-ordinated response to background or light, but on the whole they retained an almost complete range of melanophore activity. Table 5, no. 4, shows that this was not invariably the case. In some specimens the melanophores were relatively active, whilst others only occasionally expanded or contracted their melanophores.

No consistent primary response could be detected with the elimination of the ocular responses, but some trace of diurnal rhythm was still apparent.

As with intact toads, water was without effect on the activity of the melanophores.

(*c*) *Chromatic responses of hypophysectomized toads*

As previous workers have found, the removal of the anterior and posterior lobes of the pituitary resulted in a loss of all chromatic response, the melanophores

Table 3. *June: temperature* 18·21° C.

♂'s.	Black illum. dry	White illum. dry				White illum. dry	Black illum. dry			
		1 hr.	2 hr.	4hr.	6 hr.		1 hr.	2 hr.	4 hr.	6 hr.
μ1	5	2+	2−	2	1·5	2	4	5	5	5
μ2	5	2+	2	2	1·5	2−	3+	5	5	4·5
μ3	5	2·5	2+	2+	1·5	1·5	4·5	5	5	5
μ4	5	2	2	2	3+	2−	4·5	5	5	5
μ5	5	2+	2	2	1·5	2	4	5	5	5
μ6	5	2+	1·5	1·5	1+	2	3+	5	5	5
μ7	5	2	1·5	1·5	1·5	2	5	5	5	5
μ8	5	2+	2	2+	1·5	2	5	5	5	5
μ9	5	2+	2	2	2	2+	5−	5	5	3+
μ10	5	3	2	2	2+	1·5	4·5	5	5	5

Table 4. *January: temperature* 13·16° C. *Differences in level of illumination*

♀'s	Shady moist (≃18 hr.)	White illum. dry, 15 W.				Shady moist (≃18 hr.)	White illum. dry, 100 W.				Shady moist (≃18 hr.)	Black illum. dry, 15 W.				Shady moist (≃18 hr.)	Black illum. dry, 100 W.			
		2 hr.	4 hr.	6 hr.	8 hr.		2 hr.	4 hr.	6 hr.	8 hr.		2 hr.	4 hr.	6 hr.	8 hr.		2 hr.	4 hr.	6 hr.	8 hr.
μ1	2+	1+	1+	1+	1	2·5	1+	1	1+	1+	1+	4	5	5	5	3·5	3·5	5−	5−	5
μ2	2	1+	1+	2	1+	3·5	1+	1·5	1	1	5	5	5	5	5	3·5	5	5	5	5
μ3	3+	1+	2	3	2	4	1	2	2−	1+	4	5	5	5	5	3·5	5	5	5	5
μ4	4·5	1+	1·5	1·5	1	3	1	2	1+	1	3−	5	5	5	5	5	5	5	5	5
μ5	5	2·5	4	2+	3	5−	1+	1+	1+	2+	3−	5	5	5	5	4	5	5	5	5

remaining permanently contracted ($\mu = 1$) under all experimental conditions—light, background or moisture having no effect. (10 specimens).

With time however, there occurred a dilation of the capillaries of the foot, and in some cases a slight relaxation of the melanophores from the perfectly punctate condition. Normal moulting was also affected by the absence of the pituitary. Hypophysectomized toads had a brown tinge due to outer dead epidermal layers accumulating. This epidermis was not shed normally and sloughed off in patches when rubbed.

Table 5. *June–August. Severance of optic nerve*

♂'s	Shady moist (≮ 18 hr.)	White illum. dry (6 hr.)	White illum. ½ im. (6 hr.)	Black illum. dry (6 hr.)	Black illum. ½ im. (6 hr.)	Dark dry (6 hr.)	Dark ½ im. (6 hr.)
$\mu 1$	4·5 to 5	3 to 4	2·5 to 4	3 to 4+	2·5 to 3+	3+to 5−	2·5 to 4·5
$\mu 2$	5	2 to 5	1 to 2·5	2·5 to 4+	2 to 4	3	2+ to 3−
$\mu 3$	1·5 to 5	2 to 5	1 to 1+	1·5 to 3−	2 to 2·5	2 to 2+	1 to 5−
$\mu 4$	1	1 to 1·5	1 to 1·5	1 to 3·5	1·5	1	1
$\mu 5$	2 to 5	2 to 4·5	2 to 3	3 to 4	1+ to 2+	1+ to 5−	1 to 2+
$\mu 6$	3+ to 5	3·5 to 5	4·5 to 5	5	4 to 5	2·5 to 5	3 to 5−

Table 6. *Enucleated toads.* (*over a period of* 6 *months*)

♀'s	Shady moist (≮ 18 hr.)	White illum. (6 hr.)	Black illum. (6 hr.)	Darkness (6 hr.)
$\mu 1$	2 to 5	1+ to 5	1·5 to 5	2 to 5
$\mu 2$	2 to 5	3 to 5	4 to 5	3 to 5
$\mu 3$	2+ to 5	1·5 to 5	2− to 5	2− to 5
$\mu 4$	2 to 5	1·5 to 5	2− to 5	2 to 5
$\mu 5$	1·5 to 5	2·5 to 5	2+ to 5	2 to 5

(*d*) *Chromatic responses following the cauterization of the infundibular stalk*

Immediately following the operation the toads (36 specimens) assumed a very dark hue which gradually lightened, and after a few days gave the normal dark adapted look—the melanophores were reticulate. The reactions of the melanophores to light, background and moisture were repeatedly tested over a period of 2 months, and although the majority of specimens showed some very slight contraction at some stage over this period, it was evident that the melanophores had lost almost all their activity, remaining almost permanently expanded.

Examination of the brains after dissection, under a binocular microscope invariably showed the pituitary intact and some damage to the infundibular stalk. The cautery needle had been applied to different regions of the infundibular stalk from the optic chiasma to the tip of the infundibular stalk posterior to the pars tuberalis. The extent of the damage varied greatly from mere superficial damage over a small area to the destruction of a portion of the infundibular stalk to a considerable depth. But in all cases, irrespective of depth or position of damage of the infundibular stalk, the result was the almost permanent expansion of the melanophores.

(*e*) *Chromatic responses following the cauterization of the infundibular stalk and hypophysectomy*

This operation reduced the viability of the toads. In six animals both cauterization and hypophysectomy were performed at the same time. One survived for 8 weeks, three for 6 weeks, the others died within 2 weeks. The ability to regulate water uptake was destroyed and the animals became extremely oedematous. The melanophores were contracted, maximum range of index shown being from punctate to stellate-punctate (i.e. $\mu = 1$ to 2), although half the specimens showed no change at all, being fully pale. Exposure to illuminated backgrounds, darkness or moisture was without effect.

In animals of the second group (18 specimens) which after cauterization were allowed to recover before being hypophysectomized, the results were identical. After hypophysectomy there was a rapid patch sloughing off of a considerable thickness of epidermis. It is known that the living epidermis in cauterized frogs is considerably thicker than the normal, and it is these additional cells that appear to cornify rapidly and are shed when the skin is rubbed.

Examination of the brains under a binocular after dissection invariably showed the absence of the pituitary and fairly extensive damage to the infundibular stalk which in some cases was almost entirely absent.

Thus the observations could be summarized as follows:

(1) The intact toad responds to a white illuminated background by contracting the melanophores; to a black illuminated background by expanding them; and shows some diurnal rhythm in darkness. Moisture and immersion are apparently without effect.

(2) Blinding causes a cessation of these co-ordinated responses but does not inhibit activity of the melanonophores.

(3) Cauterization of the optic chiasma or infundibular stalk eliminates the white background response, the melanophores being expanded. Removal of the anterior and posterior lobes of the pituitary eliminates the black background response—the melanophores being contracted.

(4) When the two above procedures are combined the melanophores are again contracted.

DISCUSSION

The discussion of the chromatic behaviour of any poikilochromic species can be separated into three parts; first, of the relevant external stimuli and their appropriate receptors; secondly, of the method of integration of these stimuli by nervous or endocrine control; and finally of the functional significance of such behaviour.

Three external physical factors are recognized as significant—light, heat and moisture—and the receptors for these factors are the eyes and the extero-receptors of the skin. However, the relative importance of these external agents appears to vary in different animals.

In the toad, the normal secondary or ocular response to illumination is apparent but throughout there is a degree of variability never fully explained. When vision is destroyed either by severing the optic nerve or enucleating, this secondary response ceases. Some melanophore activity, however, remains which is apparently unrelated to the external environment. As in the frog, the primary response does not appear.

Hogben (1924) concluded that in the terrestrial species one of the most important factors affecting adult Anura is moisture, humidity promoting darkening. This has been shown to be true in the frog (Rowlands, 1950), but in the toad, moisture has no effect upon the pigmentary effector system, although the toad's skin is apparently more sensitive than that of the frog to the water-balance principle of the posterior lobe (Jørgensen, 1950). Besson (1946), commenting upon the positive effect of moisture on the melanophores of the frog, notes that Hyla, living in a drier habitat is not sensitive to humidity.

Within the limits of experimental conditions (10–20° C.) temperature had no effect upon the melanophores of the toad; thus in the toad, as in the purely aquatic *Xenopus laevis* and in *Rana pipiens*, light is the dominant, if not the sole, external agency eliciting a chromatic response.

It is generally accepted that the integration of chromatic changes in Amphibia is hormonal, and theories of colour change control involve the intervention of one or two hormones; one or both of which may fluctuate in amount. Whilst the mechanism whereby Anura respond to a light-absorbing (black) background is agreed upon—the intermedine system (Parker, 1948)—the mechanism for response to a light-scattering background has been a subject of controversy. Hogben (1931) introduced a second hormone which induces contraction, associated directly or indirectly with the pars tuberalis. However in the toad (as in the frog) cauterization of any region of the infundibular stalk causes the permanent expansion of the melanophores. Thus it may be concluded that the removal of the pars tuberalis is not essential for the elimination of the white-background response, and although damage to the infundibular stalk may render it inactive the evidence points to the interference with the nerve supply to the posterior lobe being responsible for the cessation of the white background response. When the nervous connexions between the brain and the posterior lobe are severed, the posterior lobe thus freed secretes intermedine continuously, causing the permanent expansion of the melanophores. This permanent expansion is certainly not due solely to the interruption of the optic-pituitary tracts since enucleation or the severance of the optic nerve does not inhibit all melanophore activity. The nature of this residual activity is obscure; it is apparently independent of external factors but dependent upon the pituitary, since when the posterior lobe is removed all melanophore activity ceases and persistent pallor results. This pallor, even after the preliminary removal of the white-background response by infundibular lesion has also been observed in *Rana pipiens* (Steggarda & Soderwall, 1939). If a single hormone system prevails in the Anura the resting state of the melanophores is the contracted state now achieved. This would mean that all the responses of the melanophores involve the pituitary, there being no direct response or reflex action involving the skin receptors. On the other hand, with the elimination of the posterior lobe, it may well be that the well-known contracting effect of adrenaline becomes dominant, holding the melanophores in a contracted condition. Besson (1946) found in the frog an increase in the blood-sugar of animals made pale by injection of adrenaline or by exposure to a white background, and she considers this to be an indirect proof of the intervention of adrenaline in pigmentary functioning. Thus she would consider both pituitary and adrenal secretion as fluctuating significantly in the chromatic behaviour of the intact animal. Further work is needed to determine whether or not adrenaline does fluctuate significantly in the chromatic behaviour of the toad, *Bufo bufo*, although in *B. arenarum* Stoppani (1942) was forced to conclude that adrenaline is important in the blanching of this toad.

Finally, as to the functional significance of chromatic behaviour, it is generally agreed that chromatic responses are not primarily part of an effective system of protective coloration, as in nature the conditions capable of producing chromatic change rarely operate. It has been suggested that colour change is related to conservation of moisture and temperature control, but the melanophores in the toad are not sensitive to water or to small changes in temperature. It therefore remains a possibility that chromatic behaviour is merely a by-product of some physiological state induced by the external environment. It has been observed (Rowlands, 1950) that male frogs in the breeding season lose their apparent sensitivity to water and do not darken on immersion. Further it is seen that several hours prior to moulting a toad often becomes dark irrespective of the external conditions, but presumably reflecting the peculiar internal conditions then prevailing.

SUMMARY

1. *Bufo bufo bufo* shows no melanophore response to moisture.

2. The severance of the optic nerve or the removal of the eyes causes a cessation of secondary responses to illuminated background. Some melanophore activity remains.

3. The cauterization of the optic chiasma or the infundibular stalk brings about the permanent expansion of the melanophores.

4. Hypophysectomy, and cauterization with hypophysectomy, both produce permanent contraction of the melanophores.

5. The implications of these results in relation to chromatic control are discussed.

I have to thank Prof. E. A. Spaul, University of Leeds, in whose department the work was carried out, for his constant help and advice, and for allowing access to unpublished results of work which he directed.

REFERENCES

BESSON, S. (1946). *Les Cellules Pigmentaires du Tégument externe chez la Grenouille.* Nancy. Georges Thomas.

HOGBEN, L. T. (1923). Pigmentary effector system. III. Colour responses in hypophysectomized frogs. *Proc. Roy. Soc. B,* **95**, 15.

HOGBEN, L. T. (1924). *The Pigmentary Effector System.* London: Oliver and Boyd.

HOGBEN, L. T. & GORDON, C. (1930). Studies on the pituitary. VII. *J. Exp. Biol.* **27**, 286.

HOGBEN, L. T. & SLOME, D. (1931). The pigmentary effector System. VI. The dual character of endocrine coordination in amphibian colour change. *Proc. Roy. Soc.* B, **108**, 10.

JØRGENSEN, C. B. (1950). The amphibian water economy with special regard to the effect of neuro-hypophyseal extracts. *Acta physiol. scand.* **22** (suppl. 78).

PARKER, G. H. (1948). *Animal Colour Change and their Neurohumors.* Cambridge University Press.

PARKER, G. H. & SCATTERTY, L. E. (1937). The number of neurohumors in the control of frog melanophores. *J. Cell. Comp. Physiol.* **9**, 297.

ROWLANDS, A. (1950). The influence of water and light upon the pigmentary system in the common frog. *J. Exp. Biol.* **27**, 446.

ROWLANDS, A. (1952). The influence of water and light upon the colour change of sightless frogs. *J. Exp. Biol.* **29**, 127.

STEGGARDA, F. R. & SODERWALL, A. L. (1939). The relationship of the pars tuberalis to melanophore response in Amphibia. *J. Cell. Comp. Physiol.* **13**, 21.

STOPPANI, A. O. M. (1942). Pharmacology of colour regulation in amphibia and the importance of the endocrine glands. *J. Pharmacol.* **76**, 118.

THE INFLUENCE OF DISSOLVED OXYGEN CONCENTRATION ON THE TOXICITY OF POTASSIUM CYANIDE TO RAINBOW TROUT

By KATHLEEN M. DOWNING
The Water Pollution Research Laboratory, Langley Road, Watford

(*Received* 2 *May* 1953)

INTRODUCTION

Industrial waste waters are often discharged into rivers in which the concentration of dissolved oxygen varies considerably, and it is therefore important to know how this will affect the toxicity to fish of poisons that may be present. In 1933, Southgate, Pentelow & Bassindale found that when fish were placed in a fixed volume of a solution of potassium cyanide (0·11 p.p.m. cyanide) toxicity decreased as the concentration of oxygen was increased, but that the rate at which it decreased fell off as air saturation value was approached.

The present work was undertaken to see if similar results were obtained when fish were tested in a continuous flow of water in which the concentrations of oxygen and cyanide were kept constant. Periods of survival in tests made in this way are less likely to be distorted by the accumulation of metabolic waste products and by depletion of oxygen and poison.

MATERIALS AND METHOD

Water containing the desired concentration of oxygen was prepared by mixing suitable proportions of a stream of water saturated with air with a stream which had been deoxygenated by scrubbing with nitrogen. Potassium cyanide solution was added to the combined stream at a constant rate; the poisoned water was then thoroughly mixed and delivered into an enclosed Perspex tank (156 l. capacity). Fish could be introduced into the tank and removed from it through a valve in the top.

In four tests the total variation in the content of dissolved oxygen in the test tank as determined by Winkler's method (1889), was between 0·30 and 0·45 p.p.m., but in the remainder the variation was very much less than 0·30 p.p.m. Concentration of cyanide was determined by a modification of Robbie's (1944) phenolphthalein method (accurate to within ±4%); total variation was less than 0·008 p.p.m. in all except four tests where it was between 0·013 and 0·019 p.p.m. During all tests the temperature in the test tank was within ±0·5° of 17° C., and the pH value varied between 7·8 and 8·2.

The fish used in all but one of the tests were yearling rainbow trout (mean length 13·3 cm., standard deviation 1·4 cm.), obtained at the same time from a commercial farm. They were sorted at random into batches and kept under similar conditions in separate aquaria at the temperature of the tap-water supply; this varied between 7·5 and 12·0° C. The fish destined for the control test in water containing 3·66 p.p.m. oxygen died before the test and were replaced by another batch of yearlings obtained from the same farm. Although these were slightly larger and more variable in size (mean length 14·7 cm., standard deviation 2·7 cm.) than the original stock, they served as a guide to the tolerance of rainbow trout to this concentration of oxygen.

RESULTS

A series of twenty-seven tests was made in random order to determine the effect of the concentration of oxygen on the period of survival of fish in water containing potassium cyanide; approximately twenty-six fish which had not been fed for 24 hr. were used in each test. Nine tests were made at each of three concentrations of cyanide—0·105, 0·116, and 0·155 p.p.m. cyanide. The period of survival was taken as the time from the start of the test until the fish had lost equilibrium and lain without swimming movements for 5 sec. In most tests every fish overturned, but in water containing 0·105 p.p.m. cyanide and 7·37 p.p.m. oxygen one fish remained when the test was stopped after 22 hr., and in the same concentration of cyanide and 8·92 p.p.m. oxygen three fish remained after 39 hr. The results are shown in Fig. 1. The values for percentage saturation with oxygen have been calculated from data given by Fox (1907).

In control tests in water containing no cyanide all fish survived with no signs of distress for the whole period of the experiment (48 hr.) when the water contained 3·66 p.p.m. oxygen, but when it contained only 1·11 p.p.m. oxygen all the fish turned over within 18 min.

When the data were examined graphically by the method described by Bliss (1937), it was found that the tests could be divided into three groups: (*a*) those in which the periods of survival fitted a normal, but not a log-normal, distribution curve; (*b*) those in which the periods of survival fitted a log-normal, but not a normal, distribution curve; and (*c*) a single test (with 0·116 p.p.m. cyanide and 1·63 p.p.m. oxygen) in which the periods of survival appeared to fit both a normal and a log-normal distribution. The type of distribution is shown in Fig. 1, from which it can be seen that normal distribution was characteristic of the shorter tests and log-normal distribution of the longer tests; the range of median survival times within which transition occurred was between 3·3 and 13·0 min. Standard deviations were found to increase with median survival times and ranged from 0·78 to 4·52 min. for periods of survival which were normally distributed and, for log-normal distributions, standard deviations of the logarithms of survival times ranged from 0·17 to 0·71. Median survival times and standard deviations were computed for all tests except the two in which some fish survived; for these two tests the statistics were estimated graphically (Bliss, 1937).

A change from log-normal to normal distribution of survival times has recently been observed as the period of survival of fish in solutions of cyanide was reduced from 14 to 6 min. by increasing the concentration of poison (Herbert & Downing, in preparation). In the present experiment the concentrations of cyanide used were those in which log-normal distribution of survival times has been observed when the concentration of oxygen was kept constant at a figure adequate for survival of fish (Herbert & Merkens, 1952). It would appear, therefore, that the shape of the distribution curve is determined by the period of survival rather than by the concentration of cyanide.

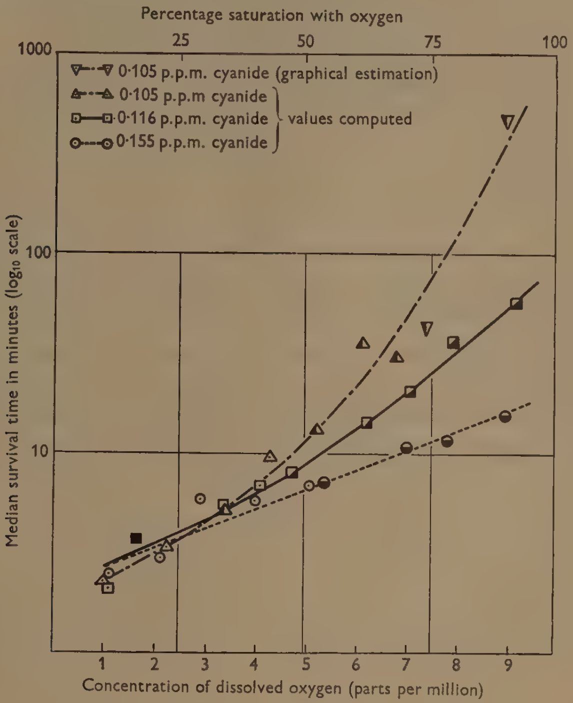

Fig. 1. Effect of concentration of oxygen on the period of survival of rainbow trout in solutions of potassium cyanide. The half-shaded points are those in which the distribution of survival time is log-normal; distributions for other points are normal; the shaded point is that in which survival time appears to fit both a normal and log-normal distribution.

The results of these tests agree with those of Southgate *et al.* (1933) in that the period of survival was prolonged by increase in the content of dissolved oxygen, but instead of the increase in the period of survival for a given increase in content of oxygen being greater in the lower concentrations of oxygen tested, the periods of survival continued to increase even when the concentration of dissolved oxygen approached the saturation value. In water containing 0·105 and 0·116 p.p.m. cyanide the effect was greater at the higher concentrations of oxygen.

SUMMARY

1. Survival times of rainbow trout in concentrations of potassium cyanide in the range 0·105–0·155 p.p.m. cyanide increased with increase in dissolved oxygen concentration between 10 and 100% of air saturation value, the effect being most marked with the lowest concentration of cyanide.

2. The rate of increase of survival time with increasing concentration of oxygen did not appear to fall off as air saturation value was approached.

3. The distributions of survival times which had median values of 3·3 min. or less were approximately normal, and those with median values of 13·0 min. or more were approximately log-normal.

This paper is published by permission of the Department of Scientific and Industrial Research.

REFERENCES

BLISS, C. I. (1937). The calculation of the time-mortality curve. *Ann. Appl. Biol.* **24**, 815.

FOX, C. J. J. (1907). On the coefficients of absorption of the atmospheric gases in distilled water and sea water. *Publ. Circ. Cons. Explor. Mer.* no. 41.

HERBERT, D. W. M. & MERKENS, J. C. (1952). The toxicity of potassium cyanide to rainbow trout. *J. Exp. Biol.* **29**, 632.

ROBBIE, W. A. (1944). An improved phenolphthalein technique for the micro-determination of cyanide. *Arch. Biochem.* **5**, 49.

SOUTHGATE, B. A., PENTELOW, F. T. K. & BASSINDALE, R. (1933). The toxicity to trout of potassium cyanide and *p*-cresol in water containing different concentrations of dissolved oxygen. *Biochem. J.* **27**, 983.

WINKLER, L. W. (1889). Die Bestimmung des im Wasser gelösten Sauerstoffs. *Ber. dtsch. chem. Ges.* **21**, 2843.

THE ACTIVITY OF SLUGS

I. THE INDUCTION OF ACTIVITY BY CHANGING TEMPERATURES

By BARBARA H. DAINTON*
Zoological Laboratory, University of Cambridge

(*Received* 30 *March* 1953)

Slug activity is associated with conditions of high humidity, and this association has, not unnaturally, led to the hitherto untested assumption that it was in some way induced by the necessarily damp conditions. The experimental examination of this phenomenon, described in this paper, has shown that certain changes in temperature, and not conditions of high humidity, induce activity. Such temperature changes occur in the situations inhabited by slugs on just those occasions when slug activity is observed in the field.

It seems desirable to define what is implied by the term 'activity'. The natural historian, when describing an animal as nocturnally active, means that it can be found at night moving about, presumably in search of food and mate, whilst during the day it is found at rest. Where this behaviour has been investigated in the laboratory (Szymanski, 1920; Gunn, 1940; Bentley, Gunn & Ewer, 1941), activity has usually been taken to mean locomotor activity because it is possible to obtain a measure of this, and it necessarily accompanies the acquisition of food and mate. In the case of slugs, where food and mate may be present in the environment during the day as well as during the night, feeding and mating occur normally only at night and follow the onset of nocturnal locomotor activity. Consequently the investigations have been directed to the study of this aspect of activity, which is regarded as the usual precursor of other activities. Unless otherwise stated, activity means locomotor activity. This usage of the term has the additional advantage of making the work directly comparable with that of other authors on different animals (Szymanski, 1920; Gunn, 1937, 1940; Bentley *et al.* 1941).

Most of the work has been done on the grey field slug *Agriolimax reticulatus* (incorrectly named *A. agrestis* by some authors), which is a pest of root crops, cereals and garden vegetables in this country. This species was chosen because it was conveniently abundant. Other species have been used occasionally, in which case this is stated in the text.

ATMOSPHERIC HUMIDITY

The activity of slugs was recorded after introduction into chambers maintained at different relative humidities. The method was similar to that used by Gunn (1937) for his investigation of the effect of atmospheric humidity on activity in the woodlouse.

---

* Present address: 16, North Parade, Leeds, 6.

The humidity chambers, which were in all essentials like those described by Gunn & Kennedy (1936), consisted of round glass dishes fitted with airtight lids. Inside, a false floor was fixed above a solution of sulphuric acid known to be in equilibrium with a certain atmospheric humidity (Wilson, 1921). Cellophane, stretched on an embroidery hoop, was substituted for the original perforated zinc floor because the slugs avoided moving over the zinc.* A rim of perforated zinc, fitting inside the hoop, prevented the slugs escaping over the edge. The chamber was left at the temperature of the experiment for at least 2 hr. to ensure that equilibrium between atmosphere and solution had been reached. This was twice the time found by Gunn to be necessary in a similar apparatus. The animals were introduced through a small hole in the lid which was covered when not in use by a greased glass slide.

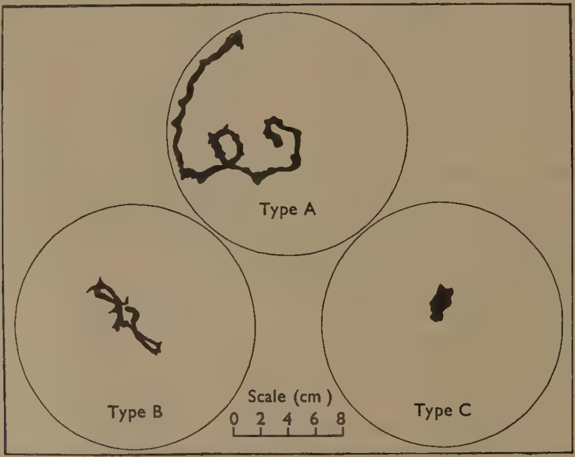

Fig. 1. Tracings of typical slug trails made in the humidity chambers.

Slugs were introduced singly into the apparatus, partly because it was felt that, in view of their wet bodies, they would, if introduced in any numbers, materially raise the humidity in the chamber, and partly because it was decided to measure the activity by the extent of the mucous trail left by the slugs instead of by direct observation. This made it possible to perform the experiments in the dark and thus eliminate any possible complication due to illumination. The animal, which was introduced through the hole in the lid, was removed after 30 min., and the trail rendered visible by immersion of the cellophane false floor in a 1% aqueous solution of Grübler's methylene blue for 5–10 min. After washing in water, the sheets of cellophane were dried and the trails examined. Introduction into the chamber is liable to induce a short period of activity due to mechanical irritation which was unavoidable. Since the likelihood of such stimulation was the same at all humidities, it could not have obscured any effect which atmospheric humidity might have had on activity.

---

* This method was suggested by Dr D. L. Gunn.

Table 1. *Types of slug trails made during* 30 *min. at different relative humidities*

Group	Temp. (° C.)	R.H. (%)	No. of trails of type		
			A	B	C
I	15·5	40	13	3	4
		100	2	8	10
II	17·5	40	12	4	4
		100	11	3	6
III	24	40	15	8	2
		100	13	11	1

130 specimens were introduced individually into chambers, half of which were at 40% and half at 100% relative humidity. After 30 min. activity had ceased, which alone showed that if the humidity was affecting the activity, the effect was extremely short-lived and quite unlike the phases of activity observed in the field. After staining, it was found possible to classify the mucous trails into three types termed A, B and C. Type A showed considerable activity, type B activity over a small area and involving much turning, and type C showed little or no activity. Tracings of typical trails are shown in Fig. 1. The number of trails of each type obtained at each humidity was counted. The results are summarized in Table 1. Three groups of experiments were performed in three rooms maintained at different approximately constant temperatures. Examination of the table shows that there was no correlation between type of trail and humidity at 17·5 or 24° C., but at 15·5° C. the activity was greater at the low humidity. A similar result can be obtained by measuring the slug trails with a map measurer and treating the distance travelled in the different groups statistically, when a significant increase in activity is only found in the dry air at 15·5° C. This increased activity following introduction into dry air is probably due to the fall in temperature resulting from loss of water by evaporation. Later experiments described in this paper show that a fall in temperature below about 21° C. stimulates activity. The actual fall necessary for stimulation varies with different individuals, which may account for the fact that the difference is not more striking, and also for the fact that no such effect was observed in group 2 at 17·5° C. (Group 3 was at 24° C., and, therefore, no such effect should have been observed.) In any case the temperature control and measurement in these experiments was hardly accurate enough, in view of later results, for deductions of this kind to be made. No further investigation was undertaken because it was apparent that this slight response to low atmospheric humidity bears no relation to activity in the field which occurs when the atmosphere is damp. It was clear from the above experiments that high atmospheric humidity does not stimulate activity. A similar result was obtained using *Arion subfuscus* instead of *Agriolimax reticulatus*.

TEMPERATURE

Slug activity is observed in the field at night and following showers or mist by day. These are both occasions, though not necessarily the only occasions, on which the temperature of the earth's surface is falling, which suggested that activity might be related to a low or falling temperature. Since slug activity occurs at all seasons (except in frosty conditions), during which the range of temperature fluctuations varies considerably, it seemed unlikely that slug activity was related to any particular temperature; but it was considered desirable, nevertheless, to start by measuring the activity of slugs at different constant temperatures because such information would clearly facilitate the interpretation of the results obtained at fluctuating temperatures.

Crozier & Pilz (1924) showed that the speed of locomotion in *Agriolimax campestris* varied directly with the temperature. This effect must be clearly distinguished from the responses described below in which inactive animals are stimulated, and the amount rather than the speed of activity is measured. In all experiments, the effect described by Crozier & Pilz was confirmed for *A. reticulatus*, the animals moving faster at high than at low temperatures.

The behaviour of the slugs was studied under conditions of constant or fluctuating temperature in an apparatus which ensured damp conditions and a constant light intensity of 40 foot-candles. Experiments described in a later paper showed that the light was not affecting the results provided 1 hr. was allowed for light adaptation after introduction into the apparatus. The use of light enabled the activity to be recorded by observation. This meant that a far greater number of animals could be employed than was possible if the activity of individual animals was recorded in the dark, using the slime-trail technique described above, or some form of mechanical recording used in a few later experiments described below.

The slugs were placed in Petri dishes which were immersed in a water-bath fitted with heating and cooling systems and, when required, with a thermostat which maintained the temperature constant to within 0·1° C. When fluctuating temperatures were in use a record was kept by making 5 min. readings from a thermometer immersed in the bath.

Three slugs were placed in each Petri dish (it was found that they did not interfere with one another), the larger side of which was lined with filter paper. This was done by placing a sheet of filter paper of slightly greater diameter than the Petri dish, over the lid which was placed upside down on a table. The slugs were placed on the filter paper and the bottom of the dish pressed into position. The Petri dishes were secured upside down to a wire frame by rubber bands, and the frame fixed 6–8 cm. below the surface of the water. The water was stirred by a stream of air bubbles which was turned off for each observation, so that the animals were readily visible through the water. The filter papers became damp because their edges protruded from the Petri dishes, whilst the dishes retained their air because their two halves were firmly held together by rubber bands. The air inside the dishes was thus conveniently maintained at 100% relative humidity. The fact that the animals were in good condition after periods in the apparatus twice as long as the duration of any experiment was taken as adequate evidence that limitation of air supply was not affecting the results.

At each observation the animals were found to be in one of three conditions: either actually moving, resting with one or more tentacles extended, or resting with tentacles retracted. The three conditions were named 'active', 'extended' and 'inactive' respectively, and the following criteria adopted for their definition. In an 'active' animal the tip of the tail could be observed to be moving; in an extended animal one or both of the dorsal pair of tentacles were visible, the animal either holding its head still or waving it from side to side; whilst in an 'inactive' animal the tentacles were withdrawn. At each observation the number of animals in each condition was recorded, thus giving an estimate of their activity. The number of animals either active or extended, and the number of active animals, were expressed as a percentage of the total numbers. In experiments involving fluctuating temperatures each reading was plotted in the form of a histogram so as to represent the condition of the animals during 5 min., the reading being taken in the middle of this period. It was found that an 'extended' animal was nearly always either about to move or settling down after a period of locomotion. Animals in such a condition would not produce either a slime trail or a mechanical record, and thus only the record of 'active' animals is strictly comparable with results obtained by the other methods of recording activity mentioned above. In the majority of experiments an apparatus holding ten dishes, and therefore thirty slugs, was used in which it took about 30 sec. to note the condition of the animals. In some experiments at constant temperature a larger apparatus holding seventy-two animals was used and the observations then took over a minute. The smaller apparatus was found to be more satisfactory for fluctuating temperatures because it was easier to control the temperature of the smaller volume of water, and because, when heating or cooling was in progress, it was clearly desirable to turn off the stream of bubbles used for stirring for as short a time as possible when observations were made.

After introduction into the apparatus the animals, even if previously light adapted, showed an initial burst of activity due to mechanical irritation. This was increased if the temperature change resulting from introduction was such as to stimulate activity. No observations were included in the results until the activity had fallen to a constant value. One hour was found to be sufficient for this in all except a very few experiments in which a further half-hour was allowed. In the majority of experiments readings were made at 5 min. intervals, though in some of the longer experiments using constant temperatures they were made at 15 or 60 min. intervals. The duration of the experiments varied from 30 min. to 4½ hr. The average reading at constant temperatures was found to be unaffected by the duration of the experiment, and in later experiments, including those involving fluctuating temperatures, 5 min. readings were made for periods of 1–3 hr.

Preliminary experiments indicated that the activity of the animals was similar at all constant temperatures, though, when they did move, they did so much more quickly at higher temperatures. The results involving changing temperature were much more striking. Activity rose sharply immediately the temperature began to fall below about 21° C., and a similar increase occurred when it was rising above this value. The increase subsided as soon as the temperature was maintained constant at any value. Temperature changes in the reverse direction had no such immediately obvious effect; as at constant temperature the activity was low when the temperature was rising below 21° C. or falling above this value.

CONSTANT TEMPERATURE

Temperatures were employed varying between 10 and 30° C. Owing to experimental difficulties, records were not made at lower temperatures, and in view of the results obtained between 10 and 30° C. it seemed unnecessary to make further attempts to obtain satisfactorily constant lower temperatures.

Table 2. *The activity of* Agriolimax reticulatus *at different constant temperatures*

Temp. (° C.)	% active animals	
	Mean	Variation
10·5	1·5	0– 6·5
15·2	2	0–10
20	1	0– 3·5
20	2	0–10
20	2	0– 6·5
24	2	0– 4
24	2	0– 5·5
30	1	0– 3·5

A series of typical experiments are summarized in Table 2, which gives the average percentage of 'active' animals recorded at different temperatures, together with the variation observed in each instance. There is very little activity at all temperatures between 10 and 30° C. and at 20° C., where the activity of three batches of animals was recorded, the range in activity is comparable with that observed in the whole series. It may thus be concluded that high activity is not associated with any particular temperature between 10 and 30° C. For reasons already stated, no extensive experiments were made below 10° C., but preliminary observations indicated that this conclusion also holds for temperatures between 4 and 10° C., and therefore throughout the whole viable range.

THE STIMULATION OF ACTIVITY BY FALLING TEMPERATURE

Since it was found impossible to repeat exactly the temperature variation obtained in any one experiment, it was necessary to plot the results of each experiment separately, and the histograms recorded here may be regarded as those made from typical experiments. In a few instances, particularly at very low temperatures, only one experiment was performed. This result can, however, be taken as conclusive, since it involved thirty animals, and in other experiments significant differences were never observed between different batches of animals

Below about 21° C. slugs respond to falling temperature by a very marked increase in activity. The response is shown, without exception, by every animal, and to surprisingly low rates of fall. Fig. 2 illustrates a series of six experiments each involving thirty animals. The rate of fall varied from 0·1 to 19° C. per hour, and in each case it was accompanied by a marked rise in activity. It should be emphasized that in each experiment all thirty animals responded, but since they did so at different time intervals, and since, once established, slug activity is intermittent, 100% activity was never recorded. At all rates of fall, the increased activity, once initiated, was maintained as long as the temperature was falling. If the rate of fall was slow, this activity fell off immediately the temperature was maintained constant. If the rate was fast the response was maintained for some time after the temperature became constant. The fall need not be sustained to induce activity. In one experiment an increase in activity was observed following a fall of 0·3° C. in 5 min. If the fall in temperature was sustained all animals, without exception, responded.

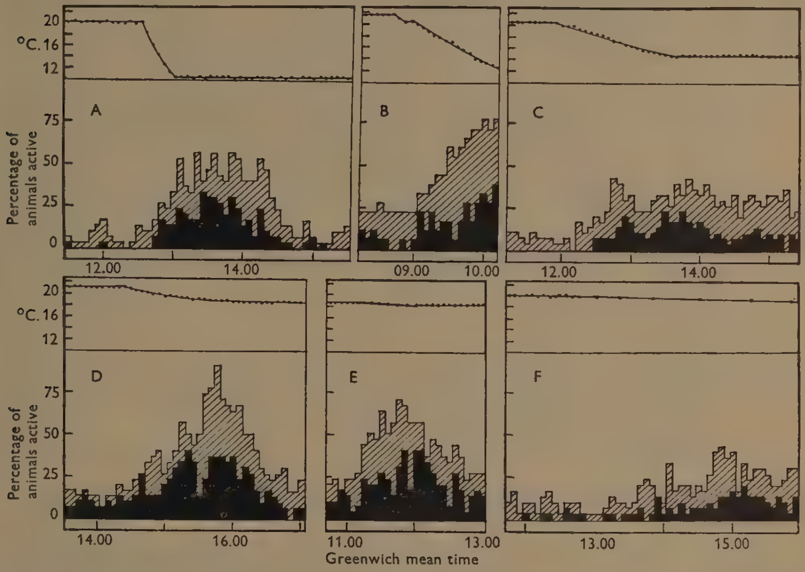

Fig. 2. The effect of falling temperature below 21° C. on activity. ■ Animals moving. ▨ Animals with extended tentacles. Average rates of fall: in A = 19° C. per hour; in B = 6·3° C. per hour; in C = 3·4° C. per hour; in D = 1·5° C. per hour; in E = 0·67° C. per hour; in F = 0·1° C. per hour.

Examination of records kept at, or near, the earth's surface (see *The Observatories Year Book*, 1939; Keen & Russell, 1921; Conway, 1936*) show that, in the slug's habitat at all seasons, the temperature falls below 21° C. only in the late afternoon, evening and early night, or following showers earlier in the day; in fact, on just those occasions when slug activity is observed in the field. The only qualification it is necessary to make is that the temperature begins to fall earlier in the afternoon than the slug activity appears. Examination of the detailed records of diurnal temperature variation among and beneath vegetation made by Conway (1936), reveal the existence of marked temperature gradients within the vegetation when the temperature first begins to fall. A study of the behaviour of slugs in such a gradient, which is described later in this paper, shows that, once active they would move up the gradient, thus counteracting the fall, and immediately coming to rest again. Only when, as shown by Conway's records, the whole of the vegetation becomes uniformly cool does slug activity of any duration appear. The time at which this occurs in Conway's records is in the evening and agrees remarkably well with the time of the appearance of slug activity in the field.

---

* Dr V. M. Conway kindly supplied me with her original complete records.

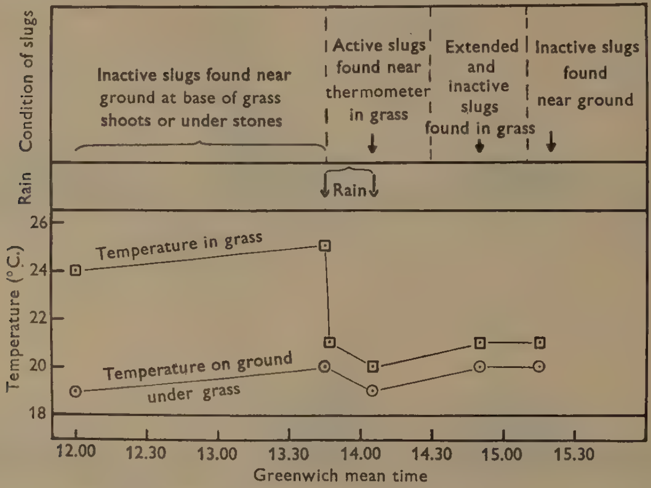

Fig. 3. Diagrammatic summary of field observation made in showery warm weather.

Fig. 3 illustrates the type of field observation which can be made in warm weather, in which marked activity was noted following a shower of rain. There is no such delay in the appearance of activity here, because the cool rain lowers the temperature of all the vegetation rapidly.

In the experiments described above the temperature never fell below 10° C. Lower temperatures do, of course, frequently occur in the slug's habitat, and exploratory experiments indicated that falling temperatures over this lower range also stimulated activity. Experimental work was not easy at these low temperatures, but a few experiments, each involving thirty animals, were made. Similar results to those described above were obtained. The burst of activity which followed the fall in temperature took longer to make its appearance than at higher temperatures. Observation of the animals suggested that this was, at any rate in large measure, due to the extreme slowness of the animals at these low temperatures. It took noticeably longer for the tentacles to be extended, and when movement did occur it was much slower.

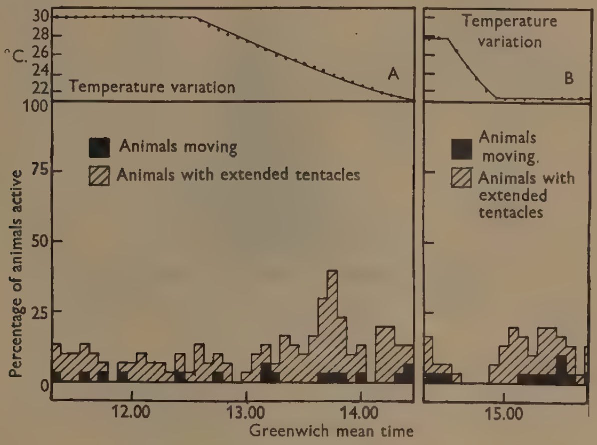

Fig. 4. The effect of falling temperature above 21° C. on activity. Rates of fall: in A = 4·2° C. per hour; in B = 12·8° C. per hour.

Above 21° C. fall in temperature has no such effect (Fig. 4), and may even reduce activity below the level observed at constant temperature.

THE EFFECT OF RISING TEMPERATURE ON ACTIVITY

Above about 21° C. rising temperature is accompanied by increased activity which falls off immediately the temperature is maintained constant as shown in Fig. 5. The response is not nearly so sensitive as that to falling temperature below 21° C., and no response was obtained to a rise of 1·7° C. per hour. Sudden increases in temperature over this range do occasionally occur in the slug's habitat. A stone or log beneath which slugs have come to rest at dawn may be suddenly exposed to the midday sun, causing the temperature to rise sharply, and in hot weather to reach proportions well above those which are lethal to slugs (30–32° C.; see Carrick, 1942). However, before such temperatures are reached, the slugs respond to the rise by becoming active, and once active they move, as described later in this paper, down the temperature gradient and thus towards some cooler, less exposed resting place.

Below 21° C. rising temperature does not stimulate activity. On the contrary, under such conditions activity is extremely low and in some experiments was practically nil (see Fig. 6). Such a rise in temperature occurs in the slug's habitat after dawn and throughout the early part of the day, a time when slug activity is

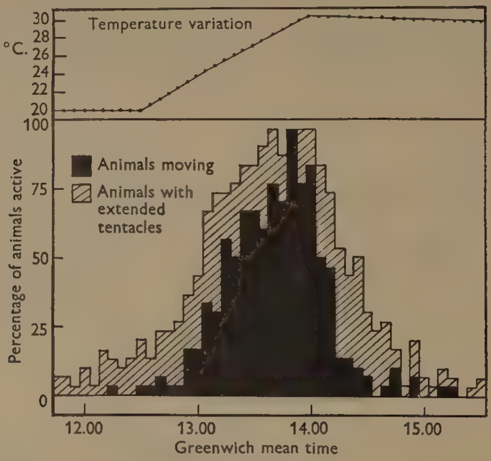

Fig. 5. The effect of rising temperature above 21° C. on activity. Average rate of rise = 6·6° C. per hour.

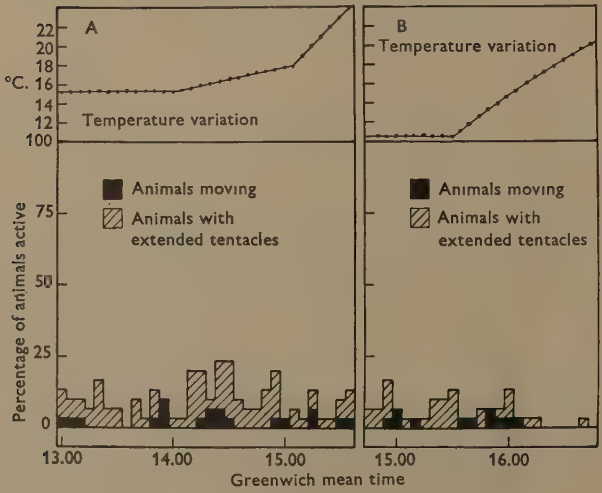

Fig. 6. The effect of rising temperature below 20–22° C. on activity.

never observed in the field except in showery weather, when the rain cooled in the upper atmosphere produces a temporary fall which stimulates a short period of activity.

THE EFFECT OF CONSTANT TEMPERATURE ON THE DIURNAL RHYTHM OF ACTIVITY AND REST

If slug activity is controlled solely by the temperature changes in the environment, the diurnal rhythm of activity and rest should disappear when the animals are kept at constant temperature. In order to obtain continuous records over several days, a mechanical form of recording the activity of individual slugs was employed. Szymanski (1920) coined the word 'aktograph' for a number of pieces of apparatus which he designed for this purpose, and one of these was adapted by Gunn & Kennedy (1936) and later by Gunn (1940). A similar, but modified aktograph was constructed and placed in a room maintained at 18° C. Records of the activity of individual slugs were kept over periods of several days.

The aktograph was essentially similar to that described by Gunn (1940). The box itself was, however, only half the size of the one in Gunn's aktograph, being 10 cm. long, 5 cm. wide and 3 cm. deep. The reduction in size was made because the slugs were not only smaller, but also much slower in motion than the cockroaches used in Gunn's experiments. It was possible for the slug to move transversely without tipping the box, but this was never observed to happen because slugs do not move in straight lines. Moreover, since the aim of the experiment was to determine when locomotory activity occurred, and not the distance traversed, the possibility that a small portion of the movement was unrecorded could be ignored. The box was made of tin and lined with paraffin wax to prevent rusting. The box in Gunn's aktograph was fitted with a false floor beneath which humidity-controlling solutions were disposed. No false floor was fitted in these experiments, but the box was lined with a double layer of thick wet filter paper which provided a damp surface for locomotion, as well as ensuring maximum humidity in the box when it was closed with a glass lid, which slid into position beneath two flanges. The lid was also lined with a double layer of wet filter paper cut slightly wider than the lid so that it projected at one side. This exposed filter paper was damped when necessary (usually daily) with water kept at the same temperature, and dropped on to it from a pipette. This could be done during a recording and caused only a very slight movement of the lever which is recognizable in the recording and can, therefore, be eliminated from the result. In this way, the slug inside the box was kept damp without upsetting the record. The base of the writing lever was made of tin and was soldered to one end of the box and an adjustable counterpoise to the other end. A long flexible celluloid lever was attached to the tin base, and this was sharpened to a point which recorded on a smoked drum. This type of lever was adopted after experiments with many other types, because it was flexible horizontally and could thus be placed firmly on the smoked drum, without greatly increasing the resistance between it and the paper, and thus reducing the sensitivity of the recording. Moreover, it was not flexible vertically and therefore gave a true record of the vertical movement of the box.

Freshly collected animals were introduced into the box one at a time, and their activity recorded under known temperature conditions for periods up to a week in duration. All records were made in continuous darkness, the glass lid of the aktograph being covered with black paper. When a constant temperature was required the aktograph was placed in a constant-temperature room at 18° C.; when a diurnally varying temperature was required it was placed in an unheated room, or beneath a shelter on the roof of the laboratory or on the grass outside. A thermograph was placed adjacent to the aktograph so that the temperature changes throughout the experiment were recorded.

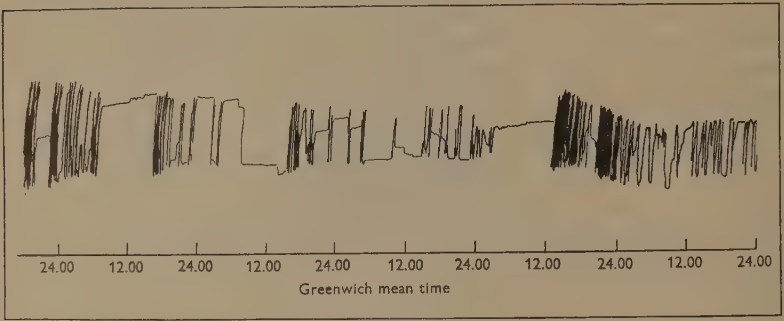

Fig. 7. Recording of the activity of one slug maintained at a constant temperature of 18° C.

Under conditions of diurnally varying temperature, the animals showed a period of activity which commenced soon after the temperature began to fall below 21° C., and terminated soon after it began to rise. When maintained at constant temperature a diurnal rhythm of activity and rest persisted for several days, but the activity was dispersed over a longer period each day until, after 4 or 5 days at constant temperature, it was more or less evenly distributed throughout the 24 hr. A record of this type is shown in Fig. 7.

If, after such a period at constant temperature, the slug is reintroduced to a diurnally varying temperature, the diurnal rhythm of activity and rest is immediately re-established, the activity occurring when the temperature is falling below 21° C. Fig. 8 shows a record made in this way.

These results are very similar to those obtained by Gunn (1940) in similar experiments on the light-controlled diurnal rhythm of activity and rest in the cockroach. In *Ptinus*, Bentley *et al.* (1941) found a similar diurnal rhythm but controlled in this case by light and temperature.

The persistence of the rhythm in an imperfect form at constant temperature is of interest, although such conditions never occur in the slug's habitat. It is necessary to postulate some form of internal stimulation in order to explain it. This internal factor may play some part in the appearance of regular nocturnal activity, but it cannot be maintained without regular diurnal temperature changes. Moreover, the rhythm gradually deteriorates and even shows some falling off during the first 24 hr. It seems, therefore, that the active phase is initiated in nature by the falling temperature and terminated when the temperature begins to rise, as shown to occur experimentally. The persistence of an imperfect rhythm at constant temperature is largely of interest in view of any light it may throw on the nervous mechanisms involved.

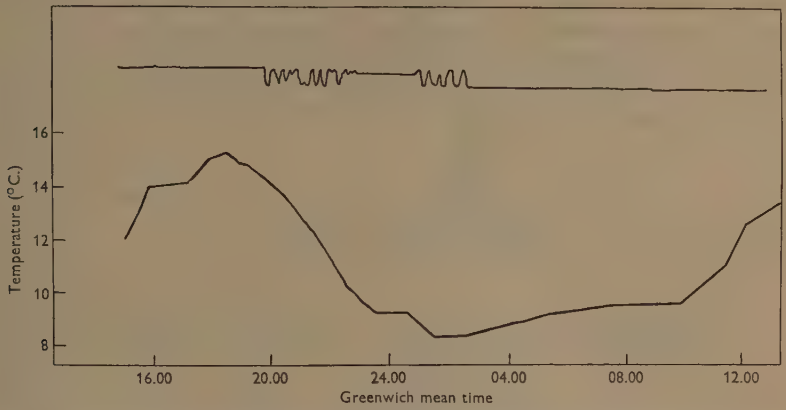

Fig. 8. Recording of the activity of one slug reintroduced into a diurnally varying temperature after 5 days at constant temperature. The thermograph recording the temperature is placed beside the apparatus, and is not near the slug, and this may account for the fact that activity does not appear to start immediately the temperature begins to fall. The cessation of activity while the temperature is still falling is probably due to loss of water in mucus production. When this was regained the temperature was constant and activity recommenced as soon as it began to fall again, and ceased once it began to rise.

TEMPERATURE PREFERENCE

It has been shown that temperature change is all important in determining activity in slugs. The slug's habitat is, moreover, situated near the earth's surface, a region of maximum temperature change and therefore of marked and changing temperature gradients. Indications of the extent of this gradient and its variation at different times of the day and at different seasons can be seen by examining the records kept on open ground at Rothamsted (Keen & Russell, 1921) and beneath vegetation at Wicken (Conway, 1936). The position in which slugs come to rest in this gradient and their behaviour towards it when active, will clearly affect the temperature changes to which they will become subjected. An investigation was, therefore, made into the behaviour of slugs in a linear temperature gradient.

The apparatus used to obtain a suitable temperature gradient between 0 and 30° C. is shown in Fig. 9. A copper trough (*t*) was made from a single sheet of copper just under 1 mm. thickness and 58 cm. long. The trough was roofed in copper for 5 cm. at each end, and each end of the roofed portion was closed with copper sheeting. This left a trough, between the two covered ends, measuring $48 \times 2{\cdot}5 \times 2{\cdot}5$ cm. The sides and floor of this trough, and of the enclosed 5 cm. at each end, were one continuous sheet of copper, thus ensuring efficient conduction. The edges were bent over to form a flat flange on which a glass lid (*gl.*) rested. Flexible metal tubing was wound seven times round each end, and was in contact with the copper. Hot water was circulated through one end and cold water through the other. In some experiments ice was packed round the cold end. The trough was placed in a wooden box packed with cotton-wool and was covered with cotton-wool during the experiments. It was illuminated by a lamp, fitted with a water filter to absorb heat rays. The resulting temperature gradient in the trough varied slightly from experiment to experiment, depending on the exact temperature of the water used, but it usually covered the range 1–30° C., although in some it did not go below 5° C. The gradient was determined by seven thermometers which passed through holes in the glass lid and which were placed so that their bulbs were just in contact with the surface over which the slugs were moving. The holes for the thermometers were 7·5 cm. apart, the end ones being 1·5 cm. from the ends of the trough. The trough was lined with graph paper on which the scales were made readily visible with Indian ink. In order to avoid the defect in such an apparatus pointed out by Gunn (1934) by which a gradient exists between the bottom and top of the apparatus as well as along it, the animals were kept within 1 cm. of the bottom by enclosing them in a 'sausage skin' of cellophane kept distended by flattened wire loops and a strip of cardboard. They were thus largely restricted in movement to a horizontal plane. The cellophane was wet to allow the animals a damp surface for locomotion.

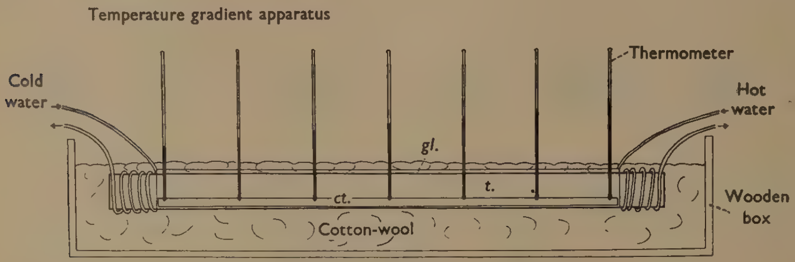

Fig. 9. Apparatus used to obtain a temperature gradient between 0 and 30° C. *t.* copper trough; *gl.* glass lid; *ct.* cellophane tube.

Twenty slugs were distributed evenly along the length of the cellophane trap and the whole dropped into the copper trough. The lid was placed on the trough and the thermometers passing through it adjusted so that they were in contact with the cellophane. No difference in temperature was detected between the top and bottom of the cellophane trap. The slugs could, and did, move freely up and down the gradient, but they could not escape from the trap and so could not move towards the lid of the trough. After 1 hr. the thermometers were read, the cotton-wool and lid removed, and the position of the head of each animal on the scale lining the trough was noted. The temperature gradient was plotted for each experiment, and from this was read the temperature at the position on the scale at which each animal was situated.

Two hundred animals were used in ten experiments, five of which were performed by day and five by night. In the daytime experiments activity was stimulated by introduction, but at the end of the experiment practically all the animals were at rest. In the night experiments they were sometimes still moving. Moreover, in the night experiments it was very hard to get an even distribution of the animals along the gradient at the start of the experiment because of the high initial activity.

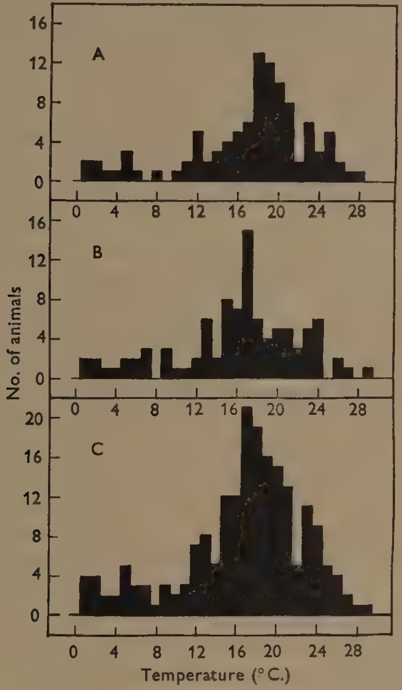

Fig. 10. Aggregation of *Agriolimax reticulatus* about a region of preferred temperature. A. Results obtained by day. B. Results obtained by night. C. Combined day and night results.

The results are summarized in Fig. 10, in which the number of animals recorded at each temperature is plotted in the form of a histogram. A is plotted from the results obtained by day when the animals were in their inactive phase, and B from those obtained at night when the animals were active. In C the two sets of results are plotted together. Both by day and night the animals aggregate about a preferred temperature of 17–18° C. The temperature appears to be slightly higher by day. There is an additional small aggregation below 8° C. The reason for this was ascertained in some further experiments in which the animals were observed through the glass roof after introduction into the apparatus. Animals introduced at very low temperatures were seen to start moving up the temperature gradient only to be overtaken by cold torpor.

These results involving a preferred temperature resemble those obtained by other authors on different animals, and in particular those obtained by Gunn & Walshe (1942) on *Ptinus tectus*, where there is also a tendency to an additional minor aggregation at very low temperatures due to cold torpor.

The movement of slugs in a temperature gradient towards a preferred temperature of 17–18° C. has a number of important results which affect their behaviour in the field.

In summer the temperature begins to fall below 21° C. some hours before activity is observed in the field. Examination of the records kept by Keen & Russell (1921) and Conway (1936) show that in summer, as would be expected, very considerable temperature gradients develop during the day near the earth's surface. Should an animal be subjected to a fall in temperature which stimulates activity, the resulting movement up the temperature gradient towards 17–18° C. (and even in very warm weather the records referred to above show that the surface of the earth where the slugs are resting does not rise above this value) would result in the slugs being subjected to a rise in temperature which, it has been shown, immediately terminates the activity. Only in the evening, when the temperature of the vegetation falls to the same value as the surface of the earth (see Conway, 1936) and there are, for a short period, no such temperature gradients, will the activity really start and the animals move out from their resting places. They are then found moving about on the surface of the vegetation. Before dawn the temperature of this vegetation may, on a clear night, fall below that of the surface of the earth beneath, in which case activity will be terminated by the movement of the animals towards the warmer earth, but since the temperature of the earth's surface is also falling the termination will only be temporary, and the activity will only finally cease at dawn when the temperature of earth and vegetation both begin to rise. At this moment the earth's surface is warmer than the vegetation and therefore the slugs come to rest near the surface, which is where they can be found during the day.

The behaviour of slugs in a temperature gradient is also very important to their survival in cold weather. Carrick (1942) has shown that, while slugs can survive exposure to one or two degrees of frost, oviposition and normal development do not occur below 5° C. In winter when the soil is warmer than the surface debris the slugs will penetrate cracks in the soil moving towards their preferred temperature, and thus avoid heavy frosts. In summer the gradient is in the opposite direction and the slugs will be led to the surface vegetation and its food supply.

The effect of vegetation will also produce horizontal gradients between open ground and that with a plant cover. In very hot and very cold weather the slugs will frequent the less exposed ground beneath vegetation by moving towards their preferred temperature.

THE EFFECT OF THE WATER CONTENT OF THE BODY IN LIMITING ACTIVITY

Howes & Wells (1934*b*) noted weight fluctuations due to variations in water content in individual slugs (*Arion ater* and *Limax flavus*), kept under certain laboratory conditions, which are similar to those found in snails (Howes & Wells, 1934*a*). The fluctuations occurred over periods of several days, and, in snails, low weight was correlated with an inactive condition (either withdrawn or aestivating), but no such correlation was made in slugs. Wells (1944) concludes, as a result of further experiments on snails, that high water content is not the immediate cause of activity which is due rather to some form of sensory stimulation, the water content simply determining whether response to such stimulation is possible. The present author has reached a similar conclusion with regard to activity in slugs. Animals of high water content show no more evidence of spontaneous activity than animals of low water content.

Slugs may lose water either by evaporation or by mucus production. Experiments were performed to determine the magnitude of the loss in each case and its effect on activity following stimulation.

The rate of loss by evaporation from the slug's body into an atmosphere of 45 % relative humidity was found to vary between 3 and 5 % of the original weight of the animal per hour, depending on the size of the slug (and therefore on the surface area/volume ratio). It was similar in all species if size was taken into consideration. The species observed included *Arion ater*, *A. subfuscus*, *Limax maximus*, *Milax sowerbyi* and *Agriolimax reticulatus*. The rates were measured in a wind tunnel (Ramsay, 1935) in moving air (347 m. per hour). Similar values were obtained in still air provided the vessel in which the slugs were dehydrated was not too small.

Experiments were performed to find whether such loss of water by evaporation resulted in reduced activity following further stimulation. Activity was induced by placing the slugs on their backs which resulted in a righting reaction and locomotion. A loss by evaporation of 4 % of the original weight of the animal had no effect on the amount of activity following this stimulation, but a loss of 17 % had a very marked effect. Loss of water by evaporation to this extent must rarely happen in nature, for slug activity occurs under damp conditions, and it would take between 3 and 5 hr. to produce such a loss by exposure to air of 45 % relative humidity, and considerably longer in damper air. Moreover, as is shown later, slugs can absorb water from damp surfaces over which they may move, and thus further reduce the rate of loss.

Slugs produce abundant mucus during locomotion. Slug mucus contains 98 % water by weight. Kunkel (1916) concluded, from a study of mucus produced by slugs in varying stages of dehydration, that the glands produce a secretion which swells rapidly on contact with water, and that in normal animals of high water content the bulk of the swelling is done by absorption of water at the expense of the animal. Mucus production, therefore, involves considerable loss of water by the animal. The present author found that specimens of *A. reticulatus* lost about

17% of their original weight through mucus production during 40 min. locomotion in a saturated atmosphere, and thus over damp surfaces.

Experiments were performed to find whether this loss of mucus during locomotion results in reduced activity in response to further stimulation. The same form of mechanical stimulation was used as in the experiments involving loss of water by evaporation. After a loss in weight of 17% due to mucus production, the slugs (*A. reticulatus*) were left resting for 20 min. and then stimulated again. This time activity was considerably reduced and the loss in weight during 40 min. was only 6% (of the original weight). A further repeat resulted in a loss of about 2%, and the activity was practically nil in spite of strong stimulation. The intervening periods of rest were included to allow the animals time for sensory adaptation or to recover from muscular fatigue. They were kept in a dry atmosphere during this time in order to prevent absorption of water from damp surfaces. It was known from the earlier experiments that the water thus lost by evaporation would be of a very small order compared with that lost in mucus production. Similar reductions in activity were obtained for *Limax maximus* and *Arion ater* following mucus production caused by stroking the animal with the handle of a scalpel. The fact that a similar reduction in activity was produced by an equivalent loss of water through evaporation suggested that the reduced activity following mucus production was due to loss of water and not loss of mucoprotein. During later investigations loss of mucus above a certain value, or of an equivalent amount of water by evaporation, was found to reduce activity in response to forms of stimulation other than the mechanical one used in these experiments.

Experiments were also performed to determine whether water loss could be made good by adsorption of water from damp surfaces, and whether, with increased water content, the ability to become active in response to suitable stimulation was regained. In all the three species investigated (see above), the animals survived a loss of 50% of their original weight and recovered their full activity, and most of their weight after some hours on damp filter paper or soil. A little weight was often lost permanently through the production of faeces.

Slugs were never observed to drink water and, though some must regularly be taken in with their food, it was clear that the bulk of this water absorption must occur through the body surface. The following experiment showed that this was possible. A specimen of *Limax maximus* weighing 9·1 g. was stimulated to produce mucus by stroking with the handle of a scalpel until it weighed only 6·6 g., after which it was in a very inactive condition. It was then suspended in a beaker of water by a thread passing through its sole, in such a position that the mouth, pneumatophone and anus were all above water-level. This meant that any gain in weight which resulted was due to absorption of water through the surface of the skin. The slug was weighed at intervals by suspending it from one arm of a balance, and it was found that practically the whole weight was regained within 2 hr. The animal was then in a very active condition. When a similarly treated animal was kept on damp soil it showed a similar recovery.

It is clear from the above experiments that water loss by evaporation must only very rarely reach proportions great enough to limit activity, whereas water loss by mucus production must frequently reach such proportions. Such water loss can, given time, be made good by absorption of water through the skin quite apart from any the animal may gain with its food. As previously mentioned, in experiments involving a number of slugs 100% activity was never recorded, even though all animals responded to the stimulus applied by becoming active; it was noted that, during a phase of activity the slug is not continuously active but pauses frequently, coming to rest and withdrawing its tentacles. It seems possible that such period of rest may occur when the water content has fallen due to mucus production, and activity does not recommence, even in the presence of the original stimulus, until the water content has risen again. The lengths of the periods of activity which occur in response to continuous stimulation will, of course, depend on the rates of mucus production and water absorption, and these will be influenced by the nature and dampness of the surface over which the slug is moving. As would be expected, the period of activity is reduced when the animal is moving over a dry surface. It should be emphasized that high water content does not, in itself, result in activity, but only enables the animal to respond to some other stimulus. This result is in agreement with the conclusion reached by Wells in the case of the snail *Helix pomatia* (Wells, 1944).

DISCUSSION

Slugs are normally active at night, but daytime activity can sometimes be observed in damp or showery weather. Since atmospheric humidity rises sharply at nightfall, and since daytime activity occurs only under damp conditions, it seemed at first sight reasonable to suppose that the activity of slugs was a response to high humidity. Experiments proved that no such response existed, and this result was confirmed by field observations which showed that while daytime activity occurs only in damp weather, it does not occur in all damp weather. Moreover, since slugs are found at rest in damp situations beneath vegetation and stones, it is hardly possible that they could be aware of humidity changes occurring in the air above, and it is also difficult to envisage, on such a wet skinned animal, any form of hygroreceptor, which could register anything but saturation. Slugs are, however, very sensitive to certain changes in temperature, and, since they live near the surface of the earth, they are subject regularly to a marked variation in this respect. They respond readily to a fall in temperature below 21° C. by becoming active, and such a fall is experienced in the evening and following showers and mist by day, in fact on just those occasions when slug activity occurs in the field. Rising temperature below 21° C. induces inactivity, and such conditions occur during the day when the slugs are found at rest beneath stones and vegetation. Thus the regular diurnal rhythm of activity and rest follows the diurnal rhythm of temperature change. Minor fluctuations may be superimposed on this diurnal temperature rhythm by precipitation of rain cooled in the upper atmosphere, and, as this results in a short fall in temperature below 21° C., the slugs respond by a short burst of activity which is terminated when the temperature begins to rise again. On continuously rainy days, when the temperature rises steadily throughout the day, no such activity occurs, though clearly conditions with respect to dampness are ideal for such activity.

Activity is also stimulated by a rise in temperature above 21° C. This response is not nearly so sensitive as that to falling temperature below 21° C., in which case a response was noted to a fall of 0·1° C. per hour. It does, however, enable the animals to escape a rapidly rising temperature which might be caused by the midday sun, and which might soon reach lethal proportions. Once active the animal will move down the temperature gradient towards some cooler resting place, and the resulting fall in temperature will soon bring it to rest, because falling temperature above 21° C., like rising temperature below 21° C., induces inactivity.

In a temperature gradient active slugs aggregate about a preferred temperature of 17–18° C. This behaviour has been shown to account for the exact time at which prolonged slug activity appears in the field. In the early afternoon when the temperature of the earth's surface first begins to fall no slug activity is apparent because, at this time, very considerable temperature gradients exist between the vegetation and the earth's surface, and any movement up such a gradient would immediately counterbalance the fall in temperature which had induced the activity, and bring the slug to rest. Only in the evening when the temperature of the vegetation falls to the same value as the surface of the earth, will prolonged activity begin. Behaviour in such a gradient also accounts for the fact that, once stimulated to activity by a rise in temperature above 21° C., the slugs move away from the region of high temperature. It also accounts, at any rate in large measure, for the fact that when slug activity subsides, the slugs come to rest beneath surface debris or vegetation and not in the open, and thereby exposed to possible desiccation or to bird predators. This response may, as described in a later paper, be augmented by a response to air movements.

Conditions of constant temperature are not experienced in the field except in heavy frost when slug activity does not occur, the slugs having retreated to warmer crevices in the soil. When maintained in the laboratory under conditions of constant temperature slugs show a persistent but immediately and steadily deteriorating diurnal rhythm of activity and rest. The persistence of such a rhythm is similar to that found by Gunn (1940) in the cockroach, and Bentley *et al.* (1941) in *Ptinus tectus*. When the activity of large numbers of slugs was measured at different constant temperatures it was found to be low in value and similar at all temperatures. The fact that slug activity is not associated with any particular temperature agrees with the field observation that it is observed at all seasons. The low level of activity found in the laboratory at constant temperature may be due to the small fluctuations necessarily inherent in any 'constant' temperature maintained by a thermostat. As already pointed out, slugs are extremely sensitive to certain types of temperature change. Or, it may be due to the initial spreading of the period of activity in the inherent rhythm described above. Such conditions are not experienced in the field where the diurnal rhythm of activity and rest is maintained by the diurnal rhythm of rising and falling temperature.

Humidity, while not responsible for the appearance of activity, may influence the amount of such activity once it occurs by determining the water content of the body. Animals of high water content are not spontaneously more active than animals of low water content, but they respond more readily to stimuli, such as mechanical irritation or certain temperature changes, which induce activity. This result is in agreement with a similar conclusion reached by Wells (1944) for the snail. Moreover, the water lost in mucus production during locomotion may temporarily reduce the water content of the body to such a level that activity ceases even in the presence of a suitable stimulus. In damp surroundings such loss is made good by absorption through the skin, and if the stimulus is still present when this reaches the necessary proportions, activity is resumed. Such an explanation may account for the observed intermittent nature of slug activity in damp conditions and under constant stimulation, both in field and laboratory.

Difficulties arise when an attempt is made to describe the temperature responses of slugs using the terminology evolved by Fraenkel & Gunn. As already stated, Crozier & Pilz (1923–4) showed that the speed of locomotion in *Agriolimax campestris* varies directly with the temperature, and this effect has been confirmed by the author for other species. Using Fraenkel & Gunn's terminology (see Fraenkel & Gunn, 1940) this is clearly a case of orthokinesis in response to temperature. If we consider the other form of orthokinesis defined by these authors, namely, the occurrence rather than the speed of locomotion, then it has been shown that there is no orthokinesis, for the activity of slugs as measured by the percentage of animals found active at any one instant is the same at all temperatures.

It has, however, been shown that rising temperature above 21° C. stimulates activity. This can also be described as an increase in activity to high and low temperatures which shows very quick adaptation. Thus it could be said that high and low temperatures result in an orthokinesis which shows almost immediate adaptation, provided the temperature is reached by the correct rise or fall, and provided this orthokinesis is distinguished from that described by Crozier & Pilz. The terminology is, however, by this time, becoming so cumbrous that it is well nigh useless. It is possible to simplify its use by making a certain change of temperature; instead of temperature, the stimulus: and thus stating that rising temperature above 21° C. or falling temperature below this value stimulates an orthokinesis. This is possibly the most satisfactory solution if this terminology must be used, but it does not distinguish this orthokinesis from that involving speed of locomotion, nor does it take any account of the effect of temperature changes in the reverse direction. In fact, an attempt to apply this terminology to these responses is certainly not satisfactory, and it is a great deal simpler and clearer to describe the responses in simple language as follows. Activity at all constant temperatures is similar and low. Rising temperature above and falling temperature below 21° C. stimulate activity. Temperature changes in the reverse direction have a depressing effect on activity and may even reduce it below the low value observed at constant temperature.

SUMMARY

1. The effect of moisture and temperature on the activity of slugs has been investigated in the laboratory and in the field.

2. Atmospheric moisture has no direct effect on activity, though it may limit the duration of activity by influencing the water content of the body. Animals of high water content are not inherently active but they respond more readily to other stimuli than animals of low water content.

3. Between 4 and 20° C. activity is induced by falling temperatures and suppressed by rising temperatures. Temperature changes as slight as 0·1° C. per hour are perceived.

4. The daily rhythm of activity and rest thus follows the normal diurnal rhythm of falling temperature by night and rising temperature by day, except when daytime mists and showers superimpose a minor fluctuation and result in daytime activity. No activity is observed on continuously wet days which are normally without such fluctuations.

5. Between 20 and 30° C. activity is induced by rising temperatures and suppressed by falling temperatures. The ecological significance of this is discussed.

6. When maintained at constant temperature slugs show a persistent but immediately and steadily deteriorating diurnal rhythm of activity and rest which could not of itself account for the regular appearance of nocturnal activity in the field.

7. In a temperature gradient slugs aggregate about a preferred temperature. The ecological significance of this is discussed.

8. The difficulty of describing these reactions in the terminology put forward by Fraenkel & Gunn (1940) is considered.

I am indebted to Dr C. F. A. Pantin, F.R.S., for his constant advice and encouragement and to Prof. J. Gray, F.R.S., for affording experimental facilities. I am also grateful to Newnham College, Cambridge, and the Department of Scientific and Industrial Research for financial aid.

REFERENCES

BENTLEY, E. W., GUNN, D. L. & EWER, D. W. (1941). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera Ptinidae), a pest of stored products. I. The daily rhythm of locomotory activity, especially in relation to light and temperature. *J. Exp. Biol.* **18**, 182.

CARRICK, R. (1942). The grey field slug, *Agriolimax agrestis* L., and its environment. *Ann. Appl. Biol.* **29**, 43.

CONWAY, V. M. (1936). Studies in the autecology of *Cladium mariscus*. II. Environmental conditions at Wicken Fen, with special reference to soil temperature and soil atmosphere. *New Phytol.* **35**, 359.

CROZIER, W. J. & PILZ, G. F. (1923–24). The locomotion of *Limax*. I. Temperature coefficient of pedal activity. *J. Gen. Physiol.* **6**, 710.

FRAENKEL, G. & GUNN, D. L. (1940). *The Orientation of Animals: Kinesis, Taxis and Compass Reactions*. Oxford.

GUNN, D. L. (1934). The temperature and humidity relations of the cockroach (*Blatter orientalis*). II. Temperature preference. *Z. vergl. Physiol.* **20**, 617.

Gunn, D. L. (1937). The humidity reactions of the wood-louse, *Porcellio scaber* (Latreille). *J. Exp. Biol.* **14**, 178.

Gunn, D. L. (1940). The daily rhythm of activity of the cockroach, *Blatter Orientalis*. I. Aktograph experiments, especially in relation to light. *J. Exp. Biol.* **17**, 267.

Gunn, D. L. & Kennedy, J. S. (1936). Apparatus for investigating the reactions of land arthropods to humidity. *J. Exp. Biol.* **13**, 450.

Gunn, D. L. & Walshe, B. M. (1942). The biology and behaviour of *Ptinus tectus* Boie (Coleoptera, Ptinidae), a pest of stored products. IV. Temperature preference. *J. Exp. Biol.* **19**, 133.

Howes, N. H. & Wells, G. P. (1934*a*). The water relations of snails and slugs. I. Weight rhythms in *Helix pomatia* L. *J. Exp. Biol.* **11**, 327.

Howes, N. H. & Wells, G. P. (1934*b*). The water relations of snails and slugs. II. Weight rhythms in *Arion ater* L. and *Limax flavus* L. *J. Exp. Biol.* **11**, 344.

Keen, B. A. & Russell, E. J. (1921). The factors determining soil temperature. *Agric. Sci.* **11**, 211.

Kunkel, K. (1916). *Zur Biologie der Lungenschnecken.* Heidelberg.

Observatories Year Book (1939). H.M. Stationery Office.

Ramsey, J. A. (1935). Methods of measuring the evaporation of water from animals. *J. Exp. Biol.* **12**, 355.

Szymanski, J. S. (1920). Aktivität und Ruhe bei Tieren und Menschen. *Z. allg. Physiol.* **18**, 105.

Wells, G. P. (1944). The water relations of snails and slugs. III. Factors determining activity in *Helix pomatia* L. *J. Exp. Biol.* **20**, 79.

Wilson, R. E. (1921). Humidity control by means of sulphuric acid solutions, with critical compilation of vapor pressure data. *J. Industr. Engng Chem.* **13**, 326.

THE ACTIVITY OF SLUGS

II. THE EFFECT OF LIGHT AND AIR CURRENTS

By BARBARA H. DAINTON*
Zoological Laboratory, University of Cambridge

(*Received* 30 *March* 1953)

Activity in slugs has been shown to occur in response to certain temperature changes (Dainton, 1954). In the description of these responses it was stated that light had no effect provided 1 hr. was allowed for light-adaptation. The experiments which led to this conclusion are described in this paper. Light initially stimulates activity in dark-adapted animals, but the response is short-lived, adaptation being complete within an hour. Preliminary observations indicated that activity was also affected by air currents, and responses to this form of stimulation are also described below.

LIGHT

Field observations alone indicate that activity is not related to light or darkness. Firstly, the normal nocturnal activity often begins before the evening light fails; secondly, daytime activity does occasionally occur; and thirdly, in the majority of situations in which slugs are found by day they are shielded from the light and are not thus subject to the fall in light intensity at eventide.

Preliminary experiments confirmed the deduction made from field observations that diurnal variations in light intensity were not the cause of diurnal variations in activity. Some indication of the condition of the animals in complete or almost complete darkness was obtained either by using an aktograph or by examining the slime trails left by the animal. Both these methods are described in the previous paper (Dainton, 1954).

Using the aktograph, the activity of individual animals was recorded throughout the day and night both under normal room conditions, in which the days were light and warm and the nights dark and cold; and at constant temperature, but subject to alternating periods of artificial illumination and darkness of about 12 hr. duration.† In neither case did the onset of nocturnal activity appear to bear any relationship to the onset of darkness, and it occurred even under conditions of artificial nocturnal illumination and daytime darkness. The appearance of the light was, however, followed by a small burst of activity apparently unrelated to the activity during the previous night. This burst of activity always subsided within an hour. After the first hour the slug was evidently adapted to the light and remained inactive throughout the day, or if it was being illuminated by night underwent its usual period of nocturnal activity at the normal time.

---

* Present address, 16, North Parade, Leeds 6.

† The author is indebted to Dr D. L. Gunn for making the first of these preliminary experiments in the aktograph which he used for his work on the diurnal rhythm of activity in the cockroach.

The activating effect of light on resting slugs was confirmed during the day by the examination of slime trails left by the animals. The effect was more marked with some individuals than others, though it seemed possible that this variation was due to insufficient time being allowed for dark-adaptation in some cases. If future experiments were to be performed in the light, it was clear that this effect of light in initially stimulating activity must be confirmed, and the time necessary for dark- and light-adaptation more accurately determined. In view of the individual variation found in the preliminary experiments, it was also obviously desirable that the experiments should be designed to include as many animals as possible.

The species *Agriolimax reticulatus* was used throughout these experiments. In all experiments the animals were kept at maximum humidity and on damp surfaces to ensure that low water content did not prevent the appearance of a response (Dainton, 1954).

An electric lamp arranged so as to produce a light intensity of 40 foot-candles over the animals was adopted as a standard illumination. In some of the later experiments a red light was substituted for this standard light. In order to eliminate the heating effect of the light, the dishes containing the animals were submerged in a water-bath maintained at constant temperature. Since it was difficult to arrange for the recording of the activity of a large number of animals kept under these conditions by either of the methods used in the preliminary experiments, it was decided to record their activity by observation at fixed intervals of time. This had the disadvantage that no indication was obtainable of conditions prior to illumination except that given by the record made immediately the light was switched on.

The apparatus used in these experiments was the same as that used in the experiments on the effect of temperature (Dainton, 1954). The slugs were kept at a constant temperature and the thermostatically controlled water-bath eliminated any heating effect of the light. A light intensity of 40 foot-candles was obtained at the lower surface of the dishes, which were immersed 8 cm. below the water surface.

After introduction into the apparatus, the animals were left in the dark for a known period. The standard light was then switched on and the condition of the animals noted immediately. The reading was taken to represent the condition of the animals in the dark. The light was left on and the observations were made at 5 min. intervals.

Since the first reading, made immediately the light was switched on, was taken as an indication of the condition of the animals in the dark, it was desirable to make it as quickly as possible and at any rate before any noticeable change in activity had occurred. It was thus necessary not to have too many animals to observe. In practice ten dishes, each containing three animals, was found to be a convenient number. The observations could then be made in about 30 sec. In some experiments seventy-two animals were used and the observations then took between 60 and 90 sec., but even then no change in the condition of the animals during this period was ever detected. The results were recorded in the form of a histogram as in the experiments on temperature. As pointed out in the previous paper

(Dainton, 1954) only the record of active animals is strictly comparable with the results of the preliminary experiments in which other methods of recording were used.

The effect of illumination on activity. Dark- and light-adaptation

Until it was realized that dark adaptation was slow, contradictory results were obtained for different animals, only some of which showed increased activity on illumination. This was similar to the result obtained in the preliminary experiments. Provided, however, sufficient time was given for dark adaptation, and about 2 hr. was found to be necessary, all animals did respond to illumination by a short burst of activity. The number of active animals fell off after 15–30 min. and light-adaptation was always complete within an hour. Such a series of experiments is illustrated in Fig. 1. In A the animals had been in the dark at 20° C. overnight. When the light was switched on next morning there was an immediate increase in activity until 46 % of the animals were either active or extended at one instant. (It should be emphasized that every animal responded, but they did so at different times, so that never more than 46 % were recorded as active or extended together.)

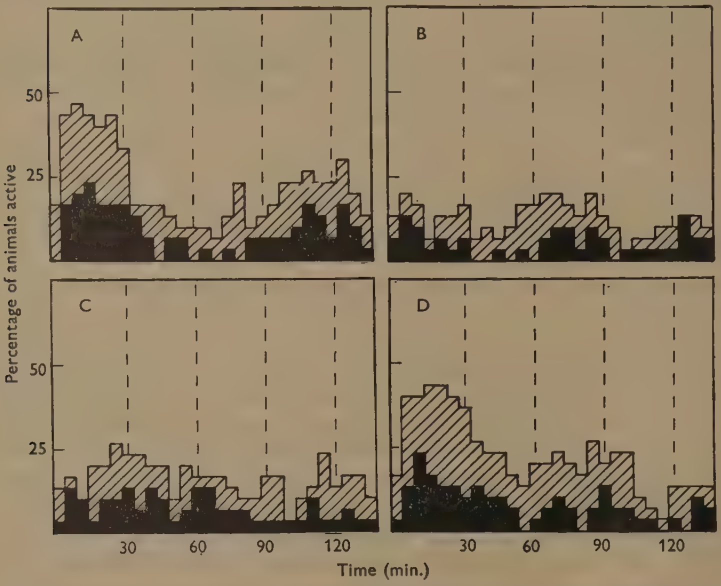

Fig. 1. The effect of illumination on the activity of 30 slugs. In A and B the animals were kept in the dark and light respectively throughout the night before illumination. In C the animals were kept in the dark for 1 hr, and in D for 2 hr., prior to illumination.

■ Animals moving. ▨ Animals with extended tentacles.

The activity then fell to a low value. In B the animals were left overnight in the light and their activity recorded at the same time next morning. No initial rise in activity was observed. In C the animals had been 1 hr. in the dark, and in D 2 hr. in the dark prior to illumination. No response was obtained after 1 hr. in the dark, but there was a marked response after 2 hr. The time necessary for dark-adaptation is therefore between 1 and 2 hr.

Examination of the histograms in Fig. 1 shows that the initial reading, which may be taken to represent the conditions of the animals in the dark prior to illumination, is of a similar value to the average reading when activity has fallen to a constant level in the light. This agrees with the preliminary experiments in suggesting that, once adaptation has occurred, illumination does not affect activity, which returns to the former low level. The reason why the activity is never recorded as zero in these experiments is discussed in an earlier paper (Dainton, 1954).

The effect of intermittent illumination

Slugs respond also to intermittent illumination. In experiments A, B and C (Fig. 2) the animals had been in the dark for at least 2 hr. prior to illumination and were thus dark-adapted. In A the slugs were illuminated continuously and in B and C the same light was switched on for readings only. In B these were taken at

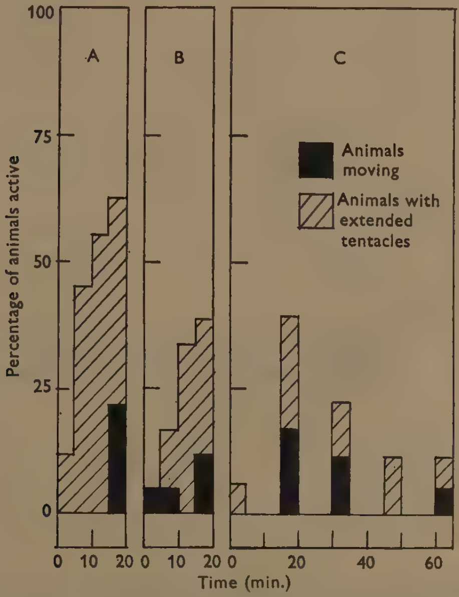

Fig. 2. The effect of constant and intermittent illumination on activity in dark-adapted specimens of *Agriolimax reticulatus*. A. Constant illumination. B. Intermittent illumination for 30 sec. of each 5 min. period. C. Intermittent illumination for 90 sec. of each 15 min. period.

5 min intervals, the light being on for 30 sec., whereas in C readings were taken at 15 min. intervals and the light was on for about 90 sec. (because there were seventy-two animals instead of thirty in the experiment and observation was therefore slower). Comparison of B and C with A shows that the slugs responded to intermittent illumination in the same way as they responded to constant illumination, and from C it can be seen that light-adaptation occurs well within the hour even with illumination for only 90 sec. of each 15 min. period.

In intermittent illumination as well as in constant illumination the activity falls to a low value following adaptation. This value was recorded for two batches of seventy-two animals, one of which was under constant illumination and the other illuminated for about 90 sec. at each 15 min. reading. The average percentage of active or extended animals recorded under constant illumination was 8·2% and under intermittent illumination 9·7%; the average percentages of active (i.e. actually moving) animals 1·3 and 0·58% respectively. In both experiments the temperature was maintained constant at 24° C. In former experiments (Dainton, 1954), involving constant conditions of temperature and illumination, a greater variation in activity was observed. There is, therefore, no significant difference between these records.

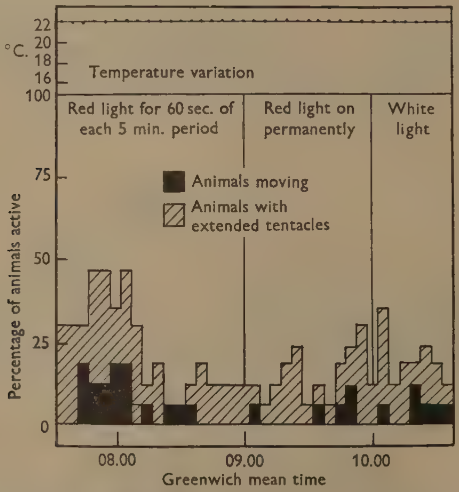

Fig. 3. The effect of red light on activity. For further explanation see text.

The effect of red light on activity

The experiments were repeated using red light. A red filter was fitted over the lamp. The filter was one which transmitted red light suitable for ordinary photographic development. Fig. 3 is made from the results of a typical experiment and shows that dark-adapted animals respond to intermittent illumination in red light by an increase in activity which falls off, adaptation being complete within 1 hr. after illumination. Once adapted to intermittent red light no increase in activity was recorded when the red light was left on permanently, or when it was replaced by white light.

The activity of seventy-two light-adapted animals was recorded in intermittent and constant red light and in constant white light, and the average percentages of animals recorded as active or extended, together with the average percentages of active animals are given in Table 1. The temperature was maintained constant at 22° C. There is, again, no significant difference in activity. The readings are higher than those quoted above (in constant and intermittent white light) because the animals in these experiments had been overnight at constant temperature (see Dainton, 1954).

Table 1. *The activity of light-adapted* Agriolimax reticulatus *under different conditions of illumination*

Type of illumination	Average % of animals recorded active or extended	Average % of animals recorded active
Intermittent, red	11·8	2·0
Constant, red	15·7	2·4
Constant, white	14·7	4·9

Discussion

Records of slug activity in the laboratory showed that the normal nocturnal activity was not necessarily associated with the onset of darkness. On the contrary, it continued under conditions of nocturnal illumination. This is in marked contrast to the results obtained by Gunn and his collaborators for the insects *Blatta orientalis* and *Ptinus tectus* (Gunn, 1940; Bentley, Gunn & Ewer, 1941). Moreover, the onset of illumination was accompanied by a short burst of activity apparently unrelated to the much longer phase of activity during the previous night. This effect of light in initially stimulating activity was confirmed by a study of the effect of illumination on slugs which were resting during the daytime in the dark.

Illumination of any individual results in a short burst of activity provided that the animal is dark-adapted. Dark-adaptation is, however, surprisingly slow, taking more than 1 hr. but less than 2 hr. Light-adaptation is more rapid and is complete well within 1 hr. Crozier & Wolf (1928–29), in a study of phototropism in the American species *Agriolimax campestris*, also found that dark-adaptation was much slower than light-adaptation. All the available evidence indicates that, once the slugs are adapted to the light, their activity is not affected by illumination, and this fact was used in the design of the experiments on the effect of temperature described in the previous paper (Dainton, 1954).

Slugs responded to intermittent illumination and to red light in exactly the same way as they responded to the standard white light used in most of the experiments.

Once adapted to red light they no longer responded to white light. No advantage is therefore gained by using red light in experiments or by turning on the light for observations only.

The significance, in the field, of the short burst of activity which follows the illumination of dark-adapted animals is not at once apparent. Clearly it does not explain the occasional occurrence of daytime activity, when slugs emerge from resting places in which they are shielded from all illumination. It may, however, be of some significance to the animal at dawn when nocturnal activity is subsiding and the short burst of activity produced by the light would decrease the likelihood of the slugs coming to rest in an illuminated, and therefore exposed, situation; in much the same way as the hygrokinesis described by Gunn (1937) in the woodlouse increases its chance of coming to rest in a damp situation. Such an effect could not, however, occur at the close of a period of daytime activity, when the slugs would be light-adapted, and in this connexion it is interesting to note that, at the close of such a period of daytime activity, inactive or extended slugs are frequently found in exposed situations.

The response described in this paper is an example of a positive photokinesis as described in the terminology of Fraenkel & Gunn (1940). As such it is to be distinguished from the phototropic responses described by Crozier & Wolf (1928–29) and involved in the circus movements which can be demonstrated in animals from which one eye has been removed. No evidence has been obtained as to whether the same receptor organs are involved, though the fact that in both responses dark-adaptation is slower than light-adaptation might perhaps suggest that this is the case. It must, however, be emphasized that the two responses have so far only been described in different species.

AIR CURRENTS

Kalmus (1942) studied the responses of slugs (*Agriolimax reticulatus*, *Arion subfuscus* and *A. hortensis*) to air currents of varying strength. He found that, if the current was so gentle as to be scarcely perceptible to the observer's cheek, the animal's tentacles, after an initial retraction, expanded towards the source of the current, the animal frequently raising its head from the table and testing the current with all four tentacles. A stronger air current, however, led to the contraction of the nearer tentacle or even the whole head, and to a subsequent sharp turning of the animal to face down wind. He suggested that the positive anemotaxis to gentle air currents may be important for the olfactory orientation of the animal.

Before Kalmus's publication appeared the present author noticed that slugs kept in the laboratory tended to avoid draughts, and experiments were performed using air currents which corresponded clearly to Kalmus's stronger currents (Dainton, 1943). The experimental investigation led to the analysis of the effect into two separate responses which are described below.

Method

Air was played on the slug from a pipette, either by use of a small rubber teat, or from the compressed air supply in the laboratory. All the experiments were repeated with air which had been saturated by bubbling it through water at room temperature. The results were always the same whether this precaution was taken or not. It was, therefore, concluded that the response was produced by the mechanical effect of the air currents, and not by a cooling or drying effect of the air. The slugs were placed on black paper on which their mucus trails were readily visible so that a permanent record of their movement was obtained. Pencil marks were made at the position of the tip of the tail at 1 min. intervals, and the application and direction of draughts was indicated with arrows. By this means speed and changes in direction could be analysed after the experiment, and attention concentrated on observing behaviour during the experiment. When active animals were required, activity was stimulated by placing them on their backs, the resulting righting reaction being followed by a short period of locomotion. In some experiments the locomotory waves on the sole of the foot were observed by placing the animals on glass and observing the sole through the glass.

All the experiments described were performed on *Agriolimax reticulatus* and *Limax maximus*. Preliminary observations indicated that similar results were obtainable on a number of other species.

Results

As already stated, two distinct reactions were obtained. One of these occurred in response to air played on the body or tail of the slug, and the other to air played on the head or tentacles.

The effect of air currents played on the body or tail

Air played on the body or tail of the slug increased the speed of locomotion or, if the animal was at rest, induced activity. Examples of the increase in speed of locomotion are given in Table 2.

Table 2. *The effect of air currents on the rate of locomotion of* Agriolimax reticulatus

Conditions	Rate of locomotion before stimulation (cm./min.)	Rate of locomotion after stimulation (cm./min.)
Water-saturated air at room temperature played on tail	3·3	4·2
Intermittent puffs of air from a pipette played on tail	2·1	2·6
Intermittent air played on tail (from compressed air supply)	1·5	3·4

This effect was also readily demonstrated by observation of the locomotory waves when the animal was moving over glass and the sole of the foot was thus visible. Stimulation of the body or tail initiated locomotory waves or increased their speed.

This response is clearly an example of an orthokinesis as defined by Fraenkel & Gunn (1940).

The effect of air currents played on the head and tentacles

If a draught of air was played across the path of an active slug just over and/or in front of the head, the animal turned away, going through the following movements. The tentacles were withdrawn, movement ceased, the head was moved to right or left, and the tentacles were extended again. This process was repeated until the tentacles eventually remained extended, the animal having turned from the region of draught, and movement began again away from the draught of air. If the draught was universal, the animal moved down stream, presumably because in this position the body provided some shelter for the head and tentacles.

Observation of the sole of an animal when moving over glass showed that air played on the head or tentacles resulted in immediate inhibition of the locomotory waves.

This response, whereby the slug turns away from the region of draught, is a klinotactic response as defined by Fraenkel & Gunn (1940).

Discussion

Both the orthokinetic and the klinotactic response described above will result in the slugs avoiding draughty situations. The klinotactic response will prevent them from entering regions of draught when more sheltered alternatives present themselves; and the orthokinetic response will increase their activity in draughty situations, and thus decrease the likelihood of the slugs coming to rest in exposed situations. These responses will clearly augment those temperature responses which lead the slugs at the close of activity into sheltered resting places (Dainton, 1954).

SUMMARY

1. Slugs are normally active at night, but this is not a response to darkness. The onset of nocturnal activity does not necessarily coincide, either in the field or in the laboratory, with the onset of darkness. On the contrary, dark-adapted, inactive animals become active on illumination.

2. The activity which follows illumination is short-lived, adaptation being complete within an hour.

3. Dark-adaptation is slower than light-adaptation and takes between 1 and 2 hr.

4. The response occurs whether illumination is constant or intermittent and in white or red light. Once adapted to red light the slugs do not respond to illumination by white light.

5. This effect of light, in initially activating the slugs, clearly cannot account for the occasional daytime activity observed in the field, when the slugs emerge from dark resting places.

6. Once the slugs are light-adapted, the activity during the daytime remains at a very low level even under continued illumination. This low level is probably the same as occurs in the dark under similar conditions.

7. Air currents played on the body or tail of the slug increase the speed of locomotion or induce activity if the animal is at rest.

8. Air currents played on the head or tentacles results in a klinotactic response whereby the animal turns from the region of draught or moves down stream.

9. These reactions must supplement the reactions in a temperature gradient which lead the slugs at the close of activity to sheltered resting places.

I am indebted to Dr C. F. A. Pantin, F.R.S., for his constant advice and encouragement, and to Prof. J. Gray, F.R.S., for affording experimental facilities. I am also grateful to Newnham College, Cambridge, and the Department of Scientific and Industrial Research for financial aid.

REFERENCES

Bentley, E. W., Gunn, D. L. & Ewer, D. W. (1941). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. I. The daily rhythm of locomotory activity, especially in relation to light and temperature. *J. Exp. Biol.* **18**, 182.

Crozier, W. J. & Wolf, E. (1928–9). Dark adaptation in *Agriolimax*. *J. Gen. Physiol.* **12**, 83 (and papers cited therein).

Dainton, B. H. (1943). Effect of air currents, light, humidity and temperature on slugs. *Nature, Lond.*, **151**, 25.

Dainton, B. H. (1954). The activity of slugs. I. The induction of activity by changing temperatures. *J. Exp. Biol.* **31**, 165.

Fraenkel, G. & Gunn, D. L. (1940). *The Orientation of Animals: Kinesis, Taxis and Compass Reactions*. Oxford.

Gunn, D. L. (1937). The humidity reactions of the wood-louse, *Porcellio scaber* (Latreille). *J. Exp. Biol.* **14**, 178.

Gunn, D. L. (1940). The daily rhythm of activity of the cockroach, *Blatta orientalis*. I. Aktograph experiments, especially in relation to light. *J. Exp. Biol.* **17**, 267.

Kalmus, H. (1942). Anemotaxis in soft-skinned animals. *Nature, Lond.*, **150**, 524.

WALLERIAN DEGENERATION IN THE RAT

THE EFFECT OF AGE ON THE CONCENTRATION OF NUCLEIC ACID AND PHOSPHOLIPID IN INTACT AND SECTIONED NERVES

By W. A. MANNELL and R. J. ROSSITER
Department of Biochemistry, University of Western Ontario, London, Canada

(*Received* 29 *June* 1953)

If a peripheral nerve is cut, that portion of the nerve distal to the point of section undergoes a series of changes characteristic of Wallerian degeneration. Logan, Mannell & Rossiter (1952*a*) showed that in the peripheral segment of the sciatic nerve of the cat degenerating after nerve section there is an increase in the concentration of nucleic acid. This change, which is probably related to the cellular proliferation that accompanies Wallerian degeneration, involves both pentosenucleic acid (PNA) and deoxypentosenucleic acid (DNA), although the increase in the two nucleic acids is disproportionate, resulting in an increase in the PNA/DNA ratio. Johnson, McNabb & Rossiter (1949*a*) and Brante (1949) showed that there is also a later decrease in the concentration of myelin lipid, related to the destruction of the myelin sheath. Mannell (1952) reported similar changes for the sciatic nerve of the rat, although in this species all the processes are considerably speeded up in time.

By observing changes in the concentration of nucleic acid and phospholipid, the study of Wallerian degeneration may be placed upon a quantitative basis. Changes in the concentration of nucleic acid give an indication of the number of cells throughout the length of a given portion of nerve. Such a determination is less time-consuming than the method of nuclear counting devised by Abercombie (1946), a method that gives the density of cell nuclei at one or more selected levels in the nerve. Changes in the concentration of phospholipid give an indication of the amount of myelin material present in the nerve, an estimate difficult to obtain by microscopic examination, for in degenerating nerve the configuration and staining properties of the myelin are quite variable.

In none of the studies mentioned above, with the exception of that of Mannell (1952), was the age of the animals controlled. Preliminary experiments by Mannell & Rossiter (1952) with two groups of rats of different ages suggested that the chemical composition of the sciatic nerve was dependent upon the age of the animal. Not only were the concentrations of nucleic acid and phospholipid greater in nerves of younger animals, but the rates at which the concentration of nucleic acid increased and the concentration of phospholipid decreased after nerve section were also much greater. That Wallerian degeneration takes place more rapidly in younger animals is stated frequently in the older writings on nerve degeneration (Ramon y Cajal, 1928, for references), but quantitative data are lacking. Since we were engaged on the study of the effect of dietary and other factors on Wallerian degeneration, it became imperative to investigate in more detail this effect of age.

In this paper is described the effect of age on the concentration of nucleic acid and phospholipid in the sciatic nerve of the rat and the effect of age on the changes that occur during Wallerian degeneration. The ages of the rats used for the study ranged from 35 to 160 days, corresponding to body weights ranging from 100 to 400 g.

METHODS

Animals

The animals were all males of the Sprague-Dawley strain. They were fed the following synthetic diet: sucrose 68%, casein 20%, corn oil 3·9%, salt mixture 4%, vitamin mixture 1%, cod-liver oil 0·1% and water 3%. The salt mixture, vitamin mixture and cod-liver oil provided adequate quantities of the minerals and vitamins known to be necessary for the rat.

Experimental

At suitable time intervals after the beginning of the experiment the sciatic nerve on one side of each of the rats was sectioned high in the thigh. The operation was performed under ether anaesthesia with full aseptic precautions. To minimize the possibility of regeneration, the two cut ends were separated as far as possible with a muscle mass. After either 8 or 16 days the animal was killed and the distal degenerating segment of nerve removed for analysis. In none of the nerves was there post-mortem evidence of regeneration. A similar length of the sciatic nerve of the opposite side was taken to serve as a control. Each nerve was rapidly cleaned of adherent fatty and epineural connective tissue and weighed on a torsion balance. The nerve was then homogenized in 3 ml. ethanol in a Potter-Elvehjem type glass homogenizer. The lipids were removed from the homogenate by extracting twice with 5 ml. ethanol, three times with 5 ml. of a 3:1 ethanol-ether mixture heated to boiling for 3 min., and once with 5 ml. ether. The acid-soluble compounds were removed with cold 10% trichloroacetic acid, and the nucleic acids were removed by extraction with 5% trichloroacetic acid for 15 min. at 90° according to the method of Schneider (1945).

Total phosphorus was determined in the combined lipid extract by the method of King (1932). The factor of 25 was used to convert lipid phosphorus to phospholipid. Total nucleic acid was determined in the hot trichloroacetic acid extract by the ultra-violet absorption method described by Logan *et al.* (1952*b*).

Recording of results

Because of the great increase in the wet weight of degenerating nerves, the results have been expressed in terms of the fresh weight of a similar length of the intact nerve of the opposite side. This method of recording results, which has been used in previous papers, is equivalent to expressing the results for the degenerating nerve in terms of the fresh weight of the nerve before it had been sectioned, i.e. at zero time.

RESULTS

Wet weight

Fig. 1 shows the correlation between the weight of the animal at time of death and the wet weight of the sciatic nerve for both intact nerves and nerves degenerating 16 days after section. As was to be anticipated, in each instance the correlation is highly significant ($P < 0{\cdot}001$). The figure also shows the great increase in the wet weight of nerves 16 days after the operation. The figures for nerves 8 days after the operation (not plotted in Fig. 1) were intermediate in value between those for the intact nerves and those for 16-day nerves.

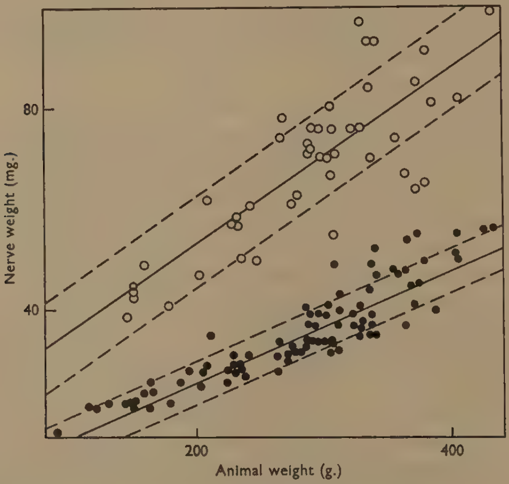

Fig. 1. Relation between nerve weight and animal weight for intact (closed circles; regression equation, $y = 0{\cdot}11X + 4$) and 16-day degenerating (open circles; regression equation, $y = 0{\cdot}17X + 19$) sciatic nerve of rat. The solid line represents the calculated regression line, and the broken lines show the standard error of the estimate of nerve weight from animal weight.

Because of this close correlation between nerve weight and animal weight, which in growing rats is closely correlated with age, nerve weight was chosen as a convenient independent variable in the study, which follows, on the effect of age on the chemical composition of the nerve.

Intact nerves

Fig. 2 shows that there is a significant ($P < 0{\cdot}001$) negative correlation between the concentration of nucleic acid and the weight of the sciatic nerve. It can also be seen from the slope of the regression line in Fig. 2 that, although the larger nerves from the older animals contain a much lower concentration of nucleic acid than the smaller nerves from the younger animals, the total quantity of nucleic acid in the nerve increases with age.

Fig. 3 shows that there is also a significant ($P < 0\cdot001$) negative correlation between the concentration of phospholipid and the weight of the nerve. In this instance the numerical value of the negative slope of the regression line is considerably less. The total quantity of phospholipid in the larger nerves thus greatly exceeds that in the smaller nerves from the younger animals. In other words, as the age of the animal increases, the total quantity of phospholipid within the nerve increases more rapidly than the total quantity of nucleic acid.

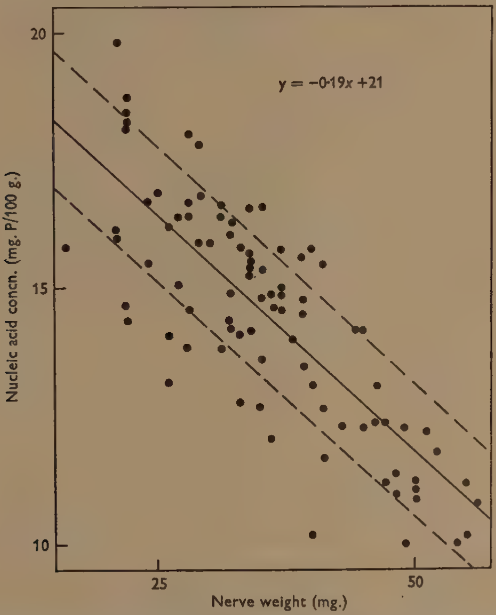

Fig. 2. Relation between the concentration of nucleic acid and nerve weight for the intact sciatic nerve of rat. The solid line represents the calculated regression line, and the broken lines show the standard error of the estimate of nucleic acid concentration from nerve weight.

Degenerating nerves

Nucleic acid. When a peripheral nerve is sectioned there is an increase in the concentration of nucleic acid in both the cat (Logan *et al.* 1952*a*) and the rat (Mannell, 1952). Fig. 4 shows that this increased concentration of nucleic acid is still negatively correlated ($P < 0\cdot01$) with the weight of the nerve both 8 days (line B) and 16 days (line *C*) after the operation. In the figure, the concentration of nucleic acid for each degenerating nerve is plotted against the weight of the corresponding intact nerve from the opposite side. On the same graph is shown the regression line for the intact nerves (line *A*), drawn from the data presented in Fig. 2.

Fig. 4 shows that the increase in the concentration of nucleic acid during the degeneration period (0–8 days) was greater in the smaller nerves from the younger animals. This is demonstrated by the significant ($P < 0{\cdot}01$) difference between the slopes of regression lines *A* and *B*. There is also a lesser, and statistically less significant ($P < 0{\cdot}05$), difference between the slopes of the regression lines *A* and *C*. These findings indicate that in the smaller nerves of the younger animals the increase in the concentration of nucleic acid, especially during the first 8 days after nerve section, is greater than that in the larger nerves of the older animals.

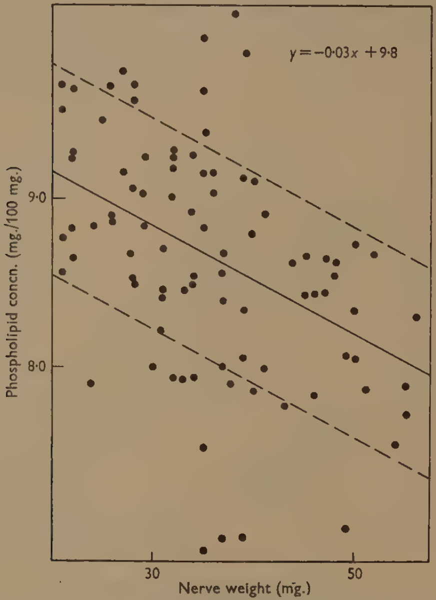

Fig. 3. Relation between the concentration of phospholipid and nerve weight for the intact sciatic nerve of rat. The solid line represents the calculated regression line, and the broken lines show the standard error of the estimate of phospholipid concentration from nerve weight.

Phospholipid. After nerve section there is a fall in concentration of phospholipid both in the cat (Logan *et al.* 1952*a*) and in the rat (Mannell, 1952). Fig. 5 shows the concentration of phospholipid plotted against the weight of the corresponding intact nerve for each animal. Line *A* is the regression line for intact nerves calculated from the data presented in Fig. 3. For these intact nerves there is a negative correlation between the concentration of phospholipid and nerve weight. However, in nerves degenerating 8 days after section, the correlation becomes positive (line *B*), with a statistically significant regression coefficient ($P < 0{\cdot}01$). The great difference between the slopes of the two regression lines (lines *A* and *B*) is due to the considerable decrease in the concentration of phospholipid in the smaller nerves from the younger animals, compared to the more modest decrease in the larger nerves from the older animals.

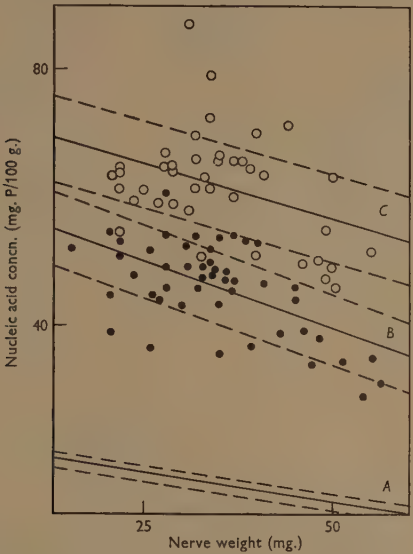

Fig. 4. Relation between the concentration of nucleic acid and nerve weight for the sciatic nerve of rat. *B* represents the regression line for 8-day degenerating nerves (closed circles; regression equation, $y = -0{\cdot}44X + 61$). *C* represents the regression line for 16-day degenerating nerves (open circles; regression equation, $y = -0{\cdot}36X + 74$). *A* is the regression line for intact nerves shown in Fig. 2.

There is no significant correlation ($P > 0{\cdot}05$) between the concentration of phospholipid in the 16-day nerves and the nerve weight (line *C*). This means that during the period 8-16 days after the operation, in contrast to the 0–8-day period, there is a considerable decrease in the concentration of phospholipid in the heavier nerves from older rats. Thus the decrease in the concentration of phospholipid occurs much earlier in nerves from the younger animals than in nerves from the older animals. Since the concentration of nucleic acid is much greater in nerves from younger animals (Fig. 2), the decrease in the concentration of phospholipid during the first 8 days of degeneration should be correlated positively with the concentration of nucleic acid in the intact nerve. Fig. 6 shows such a correlation ($P < 0{\cdot}001$).

Table 1 shows that, although the decrease in the concentration of phospholipid in the degenerating nerve during the 0–8-day period is significantly correlated with the concentration of nucleic acid in the corresponding intact nerve, it is not significantly correlated with the increase in nucleic acid that occurs during the same period. Marinesco (cited by Ramon y Cajal, 1928) suggested that the Schwann cells appearing in a nerve during Wallerian degeneration might provide enzymes that are responsible for the destruction of the lipids of the myelin sheath.

Table 1. *Coefficient of correlation (r) between the decrease in the concentration of phospholipid in the sciatic nerve of the rat 8 days after nerve section and (1) the concentration of nucleic acid in the intact nerve of the opposite side, and (2) the increase in the concentration of nucleic acid during the 8 days after the operation*

	No. animals	r	S.E. of r	P
(1) Decrease in conc. phospholipid in degenerating nerve *v.* conc. nucleic acid in intact nerve	45	0·657	0·087	<0·001
(2) Decrease in conc. phospholipid in degenerating nerve *v.* increase in conc. nucleic acid in degenerating nerve	42	0·171	0·148	>0·2

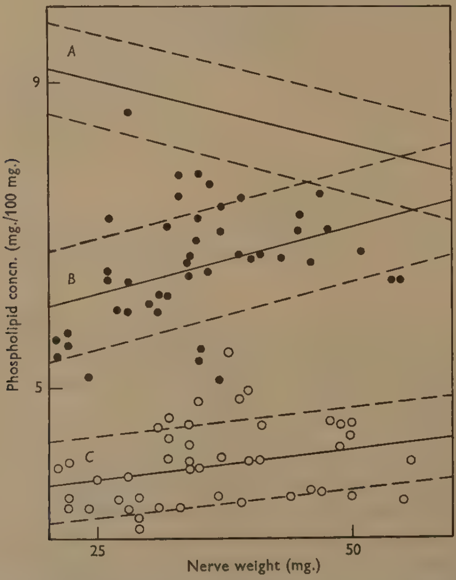

Fig. 5. Relation between the concentration of phospholipid and nerve weight for the sciatic nerve of rat. *B* represents the regression line for 8-day degenerating nerves (closed circles; regression equation, $y = 0{\cdot}04X + 5{\cdot}3$). *C* represents the regression line for 16-day degenerating nerves (open circles; regression equation, $y = 0{\cdot}02X + 3{\cdot}4$). *A* is the regression line for intact nerves shown in Fig. 3.

If this were so, the decrease in the concentration of phospholipid during the early stages of the degeneration might be expected to be correlated with the increase in the concentration of nucleic acid (representing new cells), rather than with the concentration of nucleic acid already present.

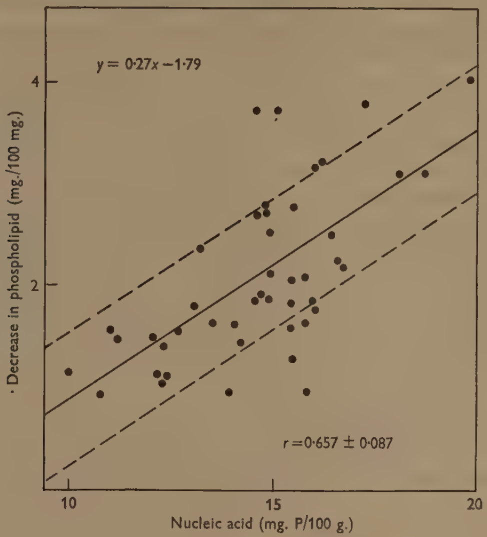

Fig. 6. Relation between the decrease in concentration of phospholipid in the sciatic nerve of rat 8-days after section and the concentration of nucleic acid in the intact nerve of the opposite side. The solid line represents the calculated regression line and the broken lines show the standard error of the estimate of decrease in phospholipid concentration from concentration of nucleic acid.

DISCUSSION

The finding that there is an increase in the wet weight of the sciatic nerve of the rat after nerve section confirms the report of Mannell (1952). An increase in the wet weight of degenerating peripheral nerve has been reported previously for a variety of species (Mott & Halliburton, 1901*a*, *b*; May, 1930; Abercrombie & Johnson, 1946; Johnson *et al.* 1949*a*).

The observation that the concentrations of both nucleic acid and phospholipid in the sciatic nerve of the rat are negatively correlated with the wet weight of the nerve is in agreement with the report of Mannell & Rossiter (1952) that in the sciatic nerves of younger animals there is a greater concentration of each of these two substances. The literature contains few reports on the effect of age on the chemical composition of peripheral nerve, but as early as 1909 Dhéré & Maurice (1909) reported that the concentration of lipid phosphorus in the peripheral nerve of dogs decreases with increasing age. These workers also presented figures for a similar decrease in the concentration of 'nucleic acid phosphorus', but, in view of the difficulties involved in the determination of nucleic acid in peripheral nerve by phosphorus estimations (Logan *et al.* 1952*b*), these figures should be treated with reserve.

The total quantity of nucleic acid in the nerve increases as the weight of the animal increases. In this regard peripheral nerve differs from the central nervous system. Mandel & Bieth (1952) have shown that for the brain of the rat, once the animal has reached the age of 16 days, there is no further increase in the total quantity of either pentosenucleic acid or deoxypentosenucleic acid.

For phospholipid, the increase in the total quantity in the nerve with increasing age is greater than that observed for nucleic acid. In the central nervous system the total quantity of phospholipid, unlike the total quantity of nucleic acid, also increases with age (Johnson *et al.* 1949*b*, for references).

In the nerves degenerating after section it seems reasonable to assume that the increase in the concentration of nucleic acid is a result of the well-known cellular proliferation (Logan *et al.* 1952*a*). Although there is some evidence (summarized by Davidson, Leslie & White, 1951) that for any one species the mean quantity of deoxypentosenucleic acid per diploid cell is constant, it should be pointed out that there is also evidence that the mean quantity of pentosenucleic acid per cell may vary considerably with the metabolic activity of the cell and a number of other extrinsic factors. In these experiments, because of the small amount of tissue available in rat nerve, the concentration of total nucleic acid (deoxypentosenucleic acid plus pentosenucleic acid) was determined. Any association between the amount of total nucleic acid and the total number of cells is only approximate.

It also seems reasonable to assume that the decrease in the concentration of phospholipid is the result of destruction of the myelin sheath. Evidence for this view has been presented by Johnson *et al.* (1949*a*, 1950). However, it should be borne in mind that phospholipid estimations, although they give a reasonable estimate of the amount of myelin material remaining in a nerve, give no indication of the degree of physical disorganization of such myelin material.

If it is granted that the increase in the concentration of nucleic acid represents a cellular proliferation and that the decrease in the concentration of phospholipid represents a destruction of the myelin sheath, both recognized features of Wallerian degeneration, it is evident that the degeneration takes place much more rapidly in younger animals. Such was to be anticipated from the older histological writings (summarized by Ramon y Cajal, 1928) and from the physiological experiments of Hines, Thomson & Lazere (1942), who reported both a more rapid degeneration and a more rapid regeneration in crushed nerves from younger animals. Results similar to those reported here have been obtained for a number of other groups of animals kept under a wide variety of dietary conditions.

SUMMARY

1. The wet weight of the sciatic nerve of the rat was significantly correlated with the body weight of the animal for both intact nerves and nerves degenerating after section. The wet weight of sectioned nerves was considerably greater than that of intact nerves.

2. The concentration of both nucleic acid and phospholipid was correlated negatively with the weight of the sciatic nerve. Hence the nerves of younger animals contained a higher concentration of each of these two substances.

3. After nerve section there was an increase in the concentration of nucleic acid and a decrease in the concentration of phospholipid. These changes took place more rapidly in smaller nerves from younger animals than in larger nerves from older animals.

Thanks are due to Mrs D. Dickey for technical assistance. The work was supported by grants from the National Research Council of Canada and the National Mental Health Grants.

REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–47.

ABERCROMBIE, M. & JOHNSON, M. L. (1946). Collagen content of rabbit sciatic nerve during Wallerian degeneration. *J. Neurol. Psychiat.* **9**, 113–18.

BRANTE, G. (1949). Studies on lipids in the nervous system with special reference to quantitative chemical determination and topical distribution. *Acta physiol. scand.* **18**, Suppl. 63, pp. 1–189.

DAVIDSON, J. N., LESLIE, I. & WHITE, J. C. (1951). The nucleic-acid content of the cell. *Lancet*, **1**, 1287–90.

DHÉRÉ, C. & MAURICE, H. (1909). Influence de l'âge sur la quantité et la répartition chimique du phosphore contenu dans les nerfs. *C.R. Acad. Sci., Paris*, **148**, 1124–5.

HINES, H. M., THOMSON, J. D. & LAZERE, B. (1942). Quantitative studies on muscle and nerve regeneration in the rat. *Amer. J. Physiol.* **137**, 527–32.

JOHNSON, A. C., MCNABB, A. R. & ROSSITER, R. J. (1949*a*). Chemical studies of peripheral nerve during Wallerian degeneration. 1. Lipids. *Biochem. J.* **45**, 500–8.

JOHNSON, A. C., MCNABB, A. R. & ROSSITER, R. J. (1949*b*). Concentration of lipids in the brain of infants and adults. *Biochem. J.* **44**, 494–8.

JOHNSON, A. C., MCNABB, A. R. & ROSSITER, R. J. (1950). Chemistry of Wallerian degeneration. A review of recent studies. *Arch. Neurol. Psychiat., Chicago*, **64**, 105–21.

KING, E. J. (1932). The colorimetric determination of phosphorus. *Biochem. J.* **26**, 292–7.

LOGAN, J. E., MANNELL, W. A. & ROSSITER, R. J. (1952*a*). Chemical studies of peripheral nerve during Wallerian degeneration. 3. Nucleic acids and other protein-bound phosphorus compounds. *Biochem. J.* **51**, 482–7.

LOGAN, J. E., MANNELL, W. A. & ROSSITER, R. J. (1952*b*). Estimation of nucleic acids in tissue from the nervous system. *Biochem. J.* **51**, 470–82.

MANDEL, P. & BIETH, R. (1952). Étude comparée du développement biochimique du cerveau chez quelques espèces de mammifères. *C.R. Acad. Sci., Paris*, **235**, 485–7.

MANNELL, W. A. (1952). Wallerian degeneration in the rat. A chemical study. *Canad. J. Med. Sci.* **30**, 173–9.

MANNELL, W. A. & ROSSITER, R. J. (1952). Effect of age on Wallerian degeneration in the rat. *Proc. Soc. Exp. Biol., N.Y.*, **80**, 262–4.

MAY, R. M. (1930). Études microchimiques sur le système nerveux: III. L'eau et les combinaisons phosphorées du nerf au cours de sa dégénérescence. *Bull. Soc. Chim. biol., Paris*, **12**, 934–53.

MOTT, F. W. & HALLIBURTON, W. D. (1901*a*). The chemistry of nerve-degeneration. *Phil. Trans.* B, **194**, 437–66.

MOTT, F. W. & HALLIBURTON, W. D. (1901*b*). The chemistry of nerve-degeneration. *Lancet*, **1**, 1077–8.

RAMON Y CAJAL, S. (1928). *Degeneration and Regeneration of the Nervous System.* London: Oxford University Press.

SCHNEIDER, W. C. (1945). Phosphorus compounds in animal tissues. 1. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. *J. Biol. Chem.* **161**, 293–303.

THE RESPIRATORY AND GLYCOLYTIC ENZYMES OF SEA-URCHIN EGGS

By MARTYNAS YČAS*

William G. Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

(*Received* 6 *July* 1953)

A number of workers have studied the carbohydrate metabolism of sea-urchin eggs and have expressed different opinions about the pathway of carbohydrate breakdown in these eggs. The choice has rested between the breakdown of hexose via the 'hexose monophosphate shunt' and a glycolytic mechanism like that in yeast and muscle, followed by oxidation via the tricarboxylic acid cycle. Rothschild (1951) has summarized the evidence on this question. In listing the evidence in favour of the latter view, Rothschild included work reported in the author's Ph.D. thesis (Yčas, 1950). Some of these results are presented in this paper. The evidence for a normal glycolytic and respiratory system has since been greatly extended by Cleland & Rothschild (1952*a*, *b*).

MATERIALS AND METHODS

Unfertilized eggs were obtained from *Strongylocentrotus purpuratus* and *Lytechinus pictus*. The animals were collected in the vicinity of the Kerckhoff Marine Biological Laboratory of the California Institute of Technology at Corona del Mar, California. Eggs were obtained either by putting the ovaries in sea water or by making the animals shed by injection of isotonic (0·55 M) KCl into the coelomic cavity (Tyler, 1949). The eggs were strained through bolting silk, washed several times in sea water, and centrifuged. Fresh homogenates were prepared by suspending the eggs in cold 1/15 M-phosphate buffer, pH 6·8, and homogenizing in a glass homogenizer. The concentration of homogenate is given as percentage by volume of eggs in the homogenate. The volume of eggs was determined by centrifuging egg suspensions in graduated centrifuge tubes. In two cases homogenates prepared from lyophilized eggs were used. O_2 uptake was measured with the conventional Warburg apparatus at 22° C.

RESULTS

Örström & Lindberg (1940) showed that immediately after fertilization of the eggs of *Paracentrotus lividus* there is a breakdown of polysaccharide material in amounts sufficient to provide substrate for the increased respiration. The breakdown of this polysaccharide, presumably glycogen, was found to be phosphorolytic. As no accumulation of phosphate esters was detected, they expressed the belief that glucose-1-phosphate was formed from glycogen and then dephosphorylated before being further metabolized by some unknown system.

---

* Present address: Pioneering Research Laboratories, U.S. Army Quartermaster Corps, Philadelphia 45, Pennsylvania.

Since such a dephosphorylation of glucose-1-phosphate appears rather improbable, an attempt was made to determine if the well-known conversion of glucose-1-phosphate to glucose-6-phosphate and fructose-6-phosphate could be demonstrated in homogenates.

The homogenate was prepared from lyophilized, unfertilized eggs of *Lytechinus pictus*. The lyophilized powder was added to 60 times its weight of distilled water, homogenized, and then dialysed against a solution of 0·5% KCl and 0·26% Na_2CO_3 for 5 hr. in the cold. Mg^{++}, cysteine (Najjar, 1948) and the potassium salt of glucose-1-phosphate were added. Aliquots were taken at 0 and 90 min. after addition of substrate, hydrolysed for 15 min. in 1 N-HCl at 100° C. and inorganic phosphate determined by the method of Fiske & Subbarow (1925). Under these conditions glucose-1-phosphate is hydrolysed completely, while glucose-6-phosphate is scarcely affected (Umbreit, Burris & Stauffer, 1945). In addition, fructose was determined by the method of Roe (1943), and calculated as fructose-6-phosphate. The results are shown in Table 1.

Table 1

Time (min.)	No. 1		No. 2	
	a	*b*	*a*	*b*
0	21	1·7	24	1·5
90	12	6·0	26	1·7

No. 1, 6 ml. egg homogenate, 1 ml. 0·1 M-cysteine, 1 ml. 0·1 M-$MgCl_2$, 1 ml. solution of 8 mg. of the potassium salt of glucose-1-phosphate and 1 ml. water. No. 2 as no. 1, substrate and water additions replaced by 4·4 mg. glucose and 25 μmoles KH_2PO_4 in 2 ml. *a*, μmoles of inorganic phosphate found after 15 min. hydrolysis in 1 N-HCl at 100°. *b*, μmoles fructose-6-phosphate. Temperature 23° C.

With the same homogenate and fructose-6-phosphate there is a rapid disappearance of substrate and conversion of the phosphate into a less easily hydrolysable form, presumably glucose-6-phosphate (Table 2). One ml. of water containing approximately 5 mg. of fructose-6-phosphate was added to 4 ml. of homogenate. The reaction was stopped by addition of 5 ml. of 10% trichloracetic acid to the samples at 0 and at 7 min. Phosphate was determined on aliquots after 0 and 180 min.

Table 2

Time (min.)	Fructose-6-phosphate (μmoles)	0 min. phosphate (μmoles)	180 min. phosphate (μmoles)
0	21	1·8	16
7	9·2	5·3	11

Disappearance of added fructose-6-phosphate in an egg homogenate. Temperature 23° C.

hydrolysis, and fructose as in the previous experiment. Under these conditions fructose-6-phosphate is hydrolysed completely and glucose-6-phosphate about 10% (Umbreit *et al.* 1945).

A decrease in fructose, estimated as fructose-6-phosphate, and in phosphate present after hydrolysis for 180 min. was observed, although the disappearance of fructose, when measured directly, is greater than that of the 180 min. phosphate.

These results are compatible with the assumption that the initial metabolism of hexose proceeds according to the classical Embden-Meyerhof sequence.

Lindberg & Ernster (1948) failed to find any accumulation of triosephosphate in egg homogenates incubated with iodoacetate; nor was the oxidation of glucose by these homogenates decreased by iodoacetate. They concluded that aldolase and triosephosphate dehydrogenase do not function in sea-urchin egg respiration.

Using the colorimetric method of Sibley & Lehninger (1949), aldolase activity is easily demonstrable in egg homogenates (Table 3).

Table 3. *Formation of triosephosphate*

Vessel	Homogenate (ml.)	Klett colorimeter reading
1	0·1	25
2	0·1	27
3	0·2	50
4	0·2	52
5	0·4	96
6	0·4	91
7	0·8	145
8	0·8	145

Composition of incubated mixture: 1 ml. glycylglycine buffer 0·1 M, pH 8·6, 0·3 ml. 1,6-fructose-diphosphate 20 mg./ml., 0·2 ml. 0·56 M-hydrazine, homogenate (100 mg. of lyophilized eggs of *Lytechinus pictus* in 6 ml. buffer) as above, and water to make to volume of 2·5 ml. Incubated 30 min. at 26° C., reaction stopped by addition of trichloracetic acid and triosephosphate determined by method of Sibley & Lehninger (1949). Blank had 0·8 ml. of homogenate, substrate added after trichloracetic acid.

The reaction of enzymes with iodoacetate is known to be rather slow. As the experiments of Lindberg & Ernster were conducted at 6° C., the reaction of iodoacetate with the dehydrogenase in their system may not have been sufficiently complete to show a detectable inhibition. To test this possibility the effect of pre-incubation with iodoacetate on the rate of reduction of methylene blue by egg homogenates was studied. Homogenates were pipetted into Thunberg tubes and iodoacetate added at various intervals of time before evacuation. The tubes were then evacuated simultaneously and, immediately after evacuation, methylene blue was tipped in from the side arms, the time to 90% decolorization being noted. Fig. 1 shows that iodoacetate inhibits the reduction of methylene blue by the endogenous substrate present in the homogenate and the inhibition increases with the period of pre-incubation with the inhibitor.

Using a more dilute homogenate, a reduction of methylene blue by 1,6-fructose-diphosphate and an inhibition of reduction by iodoacetate is shown in Table 4. These results provide some evidence that an iodoacetate-sensitive triosephosphate dehydrogenase is present in the eggs and that it functions in respiration. The importance of an adequate length of time for development of iodoacetate inhibition is also demonstrated.

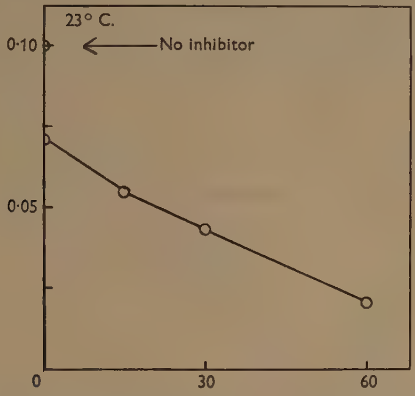

Fig. 1. Effect of pre-incubation with 0·03 M-iodoacetate on reduction of methylene blue by a 20 % homogenate of *Lytechinus pictus* eggs in M/15-phosphate buffer, pH 7·1. Ordinate: reciprocal of time in minutes till reduction. Abscissa: length of time in minutes homogenate exposed to inhibitor.

Table 4

Tube contents	Side arm	Time to decolorization (min.)
2 ml. homogenate 1 ml. water	1 ml. MB 1 ml. water	100
2 ml. homogenate 1 ml. iodoacetate	1 ml. MB 1 ml. water	>120
2 ml. homogenate 1 ml. water	1 ml. MB 1 ml. HDP	52
2 ml. homogenate 1 ml. iodoacetate	1 ml. MB 1 ml. HDP	>120

Fructose-1,6-diphosphate 0·05 M, iodoacetate 0·1 M, methylene blue 0·12 mg./ml., 5 % *Strongylocentrotus purpuratus* egg homogenate in 1/15 M-phosphate buffer, pH 7·1. Temperature 23° C. Iodoacetate added, tubes evacuated and allowed to stand 1 hr. before tipping sidearm contents.

The further metabolism of the phosphoglyceric acid formed by the oxidation of triosephosphate requires the participation of enolase. Since this enzyme is strongly inhibited by fluoride, an inhibition of the O_2 uptake of homogenates by fluoride should be demonstrable. Fig. 2 shows that such is the case. Enolase activity can be demonstrated more directly by the procedure described by Umbreit *et al.* (1945). A homogenate prepared from 0·5 g. of lyophilized eggs of *L. pictus* in 30 ml. of distilled water was dialysed in the cold against 0·5 % KCl and 0·26 %Na_2CO_3. One ml.

of this preparation was incubated with 1 ml. of 0·02 M-sodium phosphoglycerate for 10 min. at 23° C. Trichloracetic acid was then added, the incubation mixture filtered, and an aliquot treated with iodine and titrated with thiosulphate. The blank received substrate after addition of trichloracetic acid. Under these conditions, 1 ml. of the homogenate formed 0·30 mg. of phosphoenolpyruvate, which was reduced to 0·03 mg. in the presence of 0·01 M-NaF.

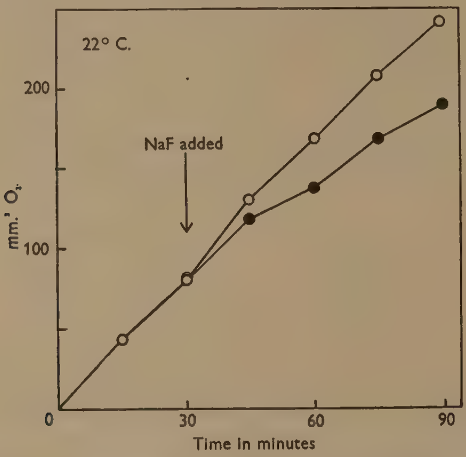

Fig. 2. Effect of NaF on the endogenous respiration of 2 ml. of a 25 % homogenate of *Strongylocentrotus purpuratus* eggs in M/15-phosphate buffer, pH 7·1. Light circles: control; dark circles: with 0·05 and 0·01 M-NaF.

Perlzweig & Barron (1928) first reported the presence of lactic acid in sea-urchin eggs, and their results have been confirmed as follows. Eggs of *Stongylocentrotus purpuratus*, placed in evacuated Thunberg tubes at 23° C. for 3 hr., were found to accumulate an average of 0·86 mg. of lactate per ml. of eggs. Controls in oxygenated sea water only contained traces of lactate. The lactate was determined by the method of Barker & Summerson (1941). A rather weak lactic dehydrogenase activity with methylene blue as a hydrogen acceptor was found to be present in homogenates.

The results obtained with iodoacetate and fluoride agree well both qualitatively and quantitatively with those reported by Cleland & Rothschild (1952*a*, *b*), even though different species and experimental conditions were used. The anaerobic formation of lactate by eggs of *S. purpuratus* (860 μg./ml. eggs/3 hr. at 23° C.) was found to be larger than in the more extensive experiments of Cleland & Rothschild with *Echinus esculentus* (300 μg./ml. eggs/4 hr. at 20° C.). Since these authors have shown that slight cytolysis greatly enhances lactate formation, and because of differences in the experimental conditions, the difference is perhaps not significant.

Although Ball & Meyerhof (1940) showed that eggs were unable to metabolize succinate and malate, implying the absence of a tricarboxylic acid cycle, Crane &

Keltch (1949) demonstrated that oxalacetate, succinate, α-ketoglutarate, glutamate and citrate are rapidly oxidized by similar homogenates. A particulate cell-free system was prepared from unfertilized *Arbacia* eggs by Keltch, Strittmatter, Walters & Clowes (1949). This system was capable of esterifying orthophosphate during the oxidation of intermediates of the tricarboxylic acid cycle. Cleland & Rothschild (1952*b*) have found that egg homogenates oxidize added pyruvate, the effect on the O_2 uptake being more marked when the formation of pyruvate from endogenous substrate is inhibited by fluoride. It is therefore probable that a tricarboxylic acid cycle does function in sea-urchin eggs.

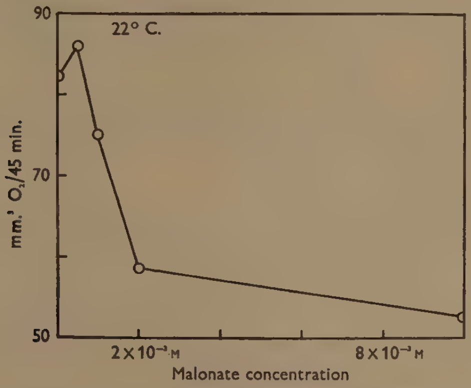

Fig. 3. Effect of malonate on the respiration of 2 ml. of a 25 % homogenate of *Strongylocentrotus purpuratus* eggs in M/15-phosphate buffer, pH 7·1.

In the present study it was found that homogenates of unfertilized eggs prepared in 1/15 M-phosphate buffer, pH 7·1, were able to oxidize citrate, α-ketoglutarate, succinate and malate as measured by an increased O_2 uptake in the presence of these acids. Since these results are not essentially different from those obtained by previous workers, details of the experiments are not given. The endogenous O_2 uptake of homogenates was found to be inhibited by malonate (Fig. 3), which is further evidence for the existence of the tricarboxylic acid cycle.

For a considerable time cytochrome was not observed in sea-urchin eggs, although cytochrome oxidase was known to be present as the terminal oxidase. In 1949 Rothschild reported that these eggs contained cytochromes *a* and b_1. This observation was easily confirmed using a hand spectroscope, although the absorption bands are rather faint. With the instrument available, the position of the cytochrome bands could not be determined with precision, but the approximate positions were at 560 and 600 mμ. No trace of the band of cytochrome *c* could be detected, even in preparations examined at the temperature of liquid air. Strong general light absorption begins at about the expected position of the cytochrome *c* band, so that failure to detect it spectroscopically cannot be considered as strong evidence for its absence. Attempts to extract cytochrome *c* from several hundred ml. of eggs by the methods of Keilin & Hartree (1937), and Rosenthal & Drabkin (1943), failed to yield any cytochrome *c*. This evidence cannot, however, be considered conclusive, as the extractability of cytochrome *c* varies in different tissues and organisms. Keilin & Hartree's method, for example, fails in the case of yeast, although cytochrome *c* is present in this organism in high concentration and can be extracted by suitable methods (Keilin, 1933).

Recently Borei (1950) has made a new attempt to detect and measure cytochrome *c* in sea-urchin eggs, using a method proposed by Paul (1950). His results indicate that at most only very small traces of cytochrome *c* can be present. While this may well be so, it should be pointed out that Paul states (personal communication, 1951) that the method is subject to hitherto unsuspected errors, a fact which was presumably not known to Borei.

As mentioned above, the absorption bands of cytochrome are faint in the sea-urchin egg, making their spectroscopic study difficult. No change could be detected with certainty in the strength of the absorption bands after aeration, anaerobiosis, or activation with sperm.

A considerable concentration of the egg cytochromes can be achieved by two different procedures. The first is essentially a modification of that used by Keilin & Hartree (1938) to prepare cytochrome oxidase from heart muscle. The procedure, as applied to eggs, was as follows: unfertilized eggs of *Lytechinus pictus* were washed several times in sea water and their jelly coats removed by allowing them to stand in sea water at a pH of 3·5 (Tyler, 1941). The eggs were then centrifuged. Throughout the rest of the treatment the preparation was kept ice cold. Twenty ml. of M/15-phosphate buffer, pH 7·1, was added to 3·5 ml. of eggs, the latter being broken up in a Waring blender. The preparation was then centrifuged for 10 min. at 3000 r.p.m. and 1 ml. of sediment came down. This fraction did not show any cytochrome bands after reduction with hydrosulphite and was discarded. The cloudy yellow supernatant was brought to pH 6 with dilute acetic acid and immediately formed a precipitate, which yielded a further 1 ml. of sediment on centrifuging for 10 min. at 3000 r.p.m. On reduction this fraction clearly showed the absorption bands of cytochromes *a* and b_1. Further acidification of the supernatant to pH 5 gave another precipitate, which, however, showed only a very weak band of cytochrome b_1.

The cytochrome components can also be concentrated by a simple centrifugation procedure. Eggs of *L. pictus* were treated as in the first procedure to remove the jelly coats and 7 ml. of eggs suspended in 13 ml. of buffer and homogenized. The homogenate was then centrifuged for 1 hr. at 10,000 *g* in a refrigerated centrifuge, the resultant precipitate being resuspended in buffer and recentrifuged. The precipitate showed strong *a* and b_1 bands when examined with the hand spectroscope. Absence of a significant amount of cytochrome *c* was demonstrated by heating the preparation to 100° C. for 1 min. This procedure destroyed the *a* and b_1 bands, as is the case in other tissues. Cytochrome *c* is stable to such treatment, but could not be demonstrated in the heated preparation after reduction with hydrosulphite.

The above results indicate that the cytochromes of sea-urchin eggs are bound to sedimentable particles, as in other tissues.

Homogenates are able both to reduce and oxidize cytochrome *c*, and both the endogenous O_2 uptake and oxidation of succinate are increased in the presence of added mammalian cytochrome *c* (Fig. 4). The apparent absence of cytochrome *c* thus poses a problem as to the mode of action of the cytochrome oxidase long known to be present in sea-urchin eggs. It would be possible to explain these results by assuming the existence of cytochrome *c* in the egg and ascribing failure to detect it to technical difficulties. It would seem best, however, to suspend judgement on this point. The nature of the component b_1 has not been elucidated. Sanborn & Williams (1950) have described a cytochrome in the tissues of the larval *Cecropia* moth, which is believed by them to show some of the properties of both cytochromes *b* and *c*, including ability to react with cytochrome oxidase. Spectroscopically this component appears identical with the cytochrome *e* recently described by Keilin & Hartree (1949) and shown by them to be as universally distributed as the normal cytochrome system. Until more work is done on the cytochromes of sea-urchin eggs, especially on the properties of the b_1 component,* the nature of the final steps of oxidation cannot be considered clarified.

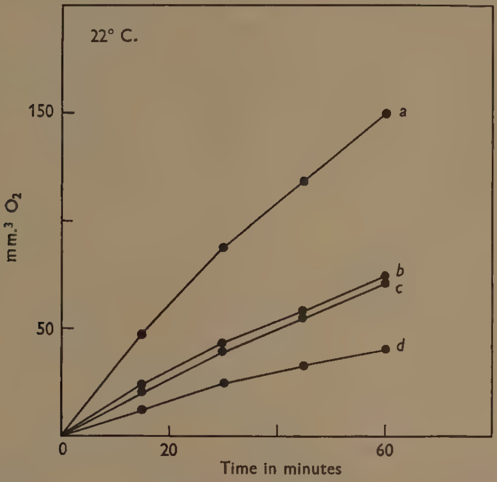

Fig. 4. Effect of cytochrome *c* and succinate on the respiration of a 25 % homogenate of *Strongylocentrotus purpuratus* eggs in M/15-phosphate buffer, pH 7·1. *a*, with 0·02 M-succinate and 5×10^{-5} M-cytochrome *c*. *b*, with 0·02 M-succinate. *c*, with 5×10^{-5} M-cytochrome *c*. *d*, no additions.

---

* Further discussion of cytochrome b_1 is to be found in Keilin & Harpley (1941) and in Rothschild (1949).

DISCUSSION

The above results show that at least a large part of the enzymatic equipment needed to metabolize carbohydrate according to the Embden-Meyerhof scheme and the tricarboxylic acid cycle is present in the sea-urchin egg, and that this is probably the major pathway of carbohydrate breakdown. The evidence for this conclusion has been greatly strengthened by Cleland & Rothschild (1952*a*, *b*). Nevertheless, it would be desirable to obtain more definitive evidence of the actual course of events *in vivo* than has yet been done. Using radioactive carbon Cohen (1951) has shown that both the usual glycolytic pathway and direct oxidation of glucose are important pathways in *Escherichia coli*. It is not impossible that the well-known changes in respiration occurring on fertilization and during development are associated with qualitative changes in metabolic pathways. The glycolytic pathway does appear, however, to be an important part of the respiratory mechanisms of the egg. An outstanding problem remains the apparent absence of cytochrome *c* and the nature and function of the b_1 component.

SUMMARY

1. Activity corresponding to phosphoglucomutase, phosphohexoisomerase, aldolase, triosephosphate dehydrogenase, enolase and lactic dehydrogenase has been demonstrated in homogenates prepared from unfertilized sea-urchin eggs (*Strongylocentrotus purpuratus* and *Lytechinus pictus*).

2. The presence of cytochromes *a* and b_1 has been confirmed. These cytochromes sediment in a relatively low centrifugal field.

3. No cytochrome *c* could be demonstrated, although cytochrome *c* is both reduced and oxidized by homogenates, and addition of cytochrome *c* increases the endogenous respiration and oxidation of succinate.

4. These results support the view that the usual glycolytic pathway operates in the sea-urchin egg and is the principal route of oxidation of carbohydrate.

I wish to express my thanks to Prof. Albert Tyler for his aid and encouragement during the course of the work reported here.

REFERENCES

BALL, E. G. & MEYERHOF, B. (1940). On the occurrence of iron-porphyrin compounds and succinic dehydrogenase in marine organisms possessing the copper blood pigment hemocyanine. *J. Biol. Chem.* **134**, 483–93.

BARKER, S. B. & SUMMERSON, W. H. (1941). The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* **138**, 535–54.

BOREI, H. (1950). Cytochromes in sea-urchin eggs. *Acta Chem. Scand.* **4**, 1607–8.

CLELAND, K. W. & ROTHSCHILD, LORD (1952*a*). The metabolism of the sea-urchin egg. Anaerobic breakdown of carbohydrate. *J. Exp. Biol.* **29**, 285–94.

CLELAND, K. W. & ROTHSCHILD, LORD (1952*b*). The metabolism of the sea-urchin egg. The oxidation of carbohydrate. *J. Exp. Biol.* **29**, 416–28.

COHEN, S. S. (1951). The synthesis of nucleic acid by virus infected bacteria. *Bact. Rev.* **15**, 131–46.

CRANE, R. K. & KELTCH, A. K. (1949). Dinitrocresol and phosphate stimulation of the oxygen consumption of a cell-free oxidative system obtained from sea-urchin eggs. *J. Gen. Physiol.* **32**, 503–9.

FISKE, L. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**, 375–400.

KEILIN, D. (1933). Cytochrome and intracellular respiratory enzymes. *Ergebn. Enzymforsch.* **2**, 239–71.

KEILIN, D. & HARPLEY, C. H. (1941). The cytochrome system in *Bacterium coli commune*. *Biochem. J.* **35**, 688–92.

KEILIN, D. & HARTREE, E. F. (1937). Preparation of pure cytochrome *c* from heart muscle and some of its properties. *Proc. Roy. Soc.* B, **122**, 298–308.

KEILIN, D. & HARTREE, E. F. (1938). Cytochrome oxidase. *Proc. Roy. Soc.* B, **125**, 171–86.

KEILIN, D. & HARTREE, E. F. (1949). Effect of low temperature on the absorption spectrum of haemoproteins, with observations on the absorption spectrum of oxygen. *Nature, Lond.*, **164**, 254–9.

KELTCH, A. K., STRITTMATTER, C. F., WALTERS, C. P. & CLOWES, G. H. A. (1949). Oxidative phosphorylation by a cell-free particulate enzyme system from unfertilized *Arbacia* eggs. *Biol. Bull., Woods Hole*, **97**, 242–3.

LINDBERG, O. & ERNSTER, L. (1948). On carbohydrate metabolism in homogenized sea-urchin eggs. *Biochim. biophys. acta*, **2**, 471–7.

NAJJAR, V. A. (1948). The isolation and properties of phosphoglucomutase. *J. Biol. Chem.* **175**, 281–90.

ÖRSTRÖM, A. & LINDBERG, O. (1940). Ueber den Kohlenhydratstoffwechsel bei der Befruchtung des Seeigeleies. *Enzymologia*, **8**, 367–84.

PAUL, K. G. (1950). The splitting with silver salts of the cysteine-porphyrin bonds in cytochrome *c*. *Acta chem. scand.* **4**, 239–44.

PERLZWEIG, W. A. & BARRON, E. S. G. (1928). Lactic acid and carbohydrate in sea-urchin eggs under aerobic and anaerobic conditions. *J. Biol. Chem.* **79**, 19–26.

ROE, J. H. (1943). A colorimetric method for the determination of fructose in blood and urine. *J. Biol. Chem.* **107**, 15–22.

ROSENTHAL, O. & DRABKIN, D. L. (1943). Spectrophotometric studies. XI. The direct micro spectrophotometric determination of cytochrome *c*. *J. Biol. Chem.* **149**, 437–50.

ROTHSCHILD, LORD (1949). The metabolism of fertilized and unfertilized sea-urchin eggs. The action of light and carbon monoxide. *J. Exp. Biol.* **26**, 100–11.

ROTHSCHILD, LORD (1951). Sperm-egg interacting substances and metabolic changes associated with fertilization. *Bioch. Soc. Symp.* no. 7, pp. 40–51.

SANBORN, R. C. & WILLIAMS, C. M. (1950). The cytochrome system in the *Cecropia* silkworm, with special reference to the properties of a new component. *J. Gen. Physiol.* **33**, 579–88.

SIBLEY, J. A. & LEHNINGER, A. L. (1949). Determination of aldolase in animal tissues. *J. Biol. Chem.* **177**, 859–72.

TYLER, A. (1941). The role of fertilizin in the fertilization of eggs of the sea-urchin and other animals. *Biol. Bull., Woods Hole*, **81**, 190–204.

TYLER, A. (1949). A simple, non-injurious method for inducing repeated spawning of sea-urchins and sand-dollars. *Collect. Net*, **19**, 19–20.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1945). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*. Minneapolis, Minnesota: Burgess Publishing Co.

YČAS, M. F. (1950). Studies on the respiratory enzymes of sea-urchin eggs. Thesis, California Institute of Technology, Pasadena, California.

ON THE DRINKING OF SOIL CAPILLARY WATER BY SPIDERS

By D. A. PARRY*

The Zoological Laboratory, University of Cambridge

(*Received* 22 *September* 1953)

INTRODUCTION

It is known or suspected that small arthropods and other animals which live in or on the soil, absorb soil moisture through their skins. I am not aware that it has hitherto been realized that some animals can *drink* soil capillary water, even against quite considerable suctions. This paper contains an account of the facility in two species of lycosid spider, and brings to light some factors upon which it depends. The spiders used were *Tarentula barbipes* (Sundevall), one of the larger British lycosids; and *Lycosa radiata* (Latreille), which is common in the south of France.

METHODS

Tarentula barbipes. The supply of animals from nature was supplemented by those bred in the laboratory. The spiders were kept separately in glass-covered 3 in. flower-pots containing soil which was kept moist by standing the pots in a large tray of water periodically replenished. Resting on the soil in each pot was a shallow waxed dish containing dry soil on which the spider normally lived. Heat and light were provided by a 3·6 W. electric bulb whose leads ran through a hole drilled through the pot. For food, drosophila, blow-fly grubs and cockroaches would all be taken provided they were not much larger than the spider itself. Mature animals were put into larger versions of the same type of cage, where the females could make the burrows within which the egg cocoons were formed; and the young were separated shortly after hatching.

Lycosa radiata was obtained in sufficient numbers from the field and has not yet been bred in the laboratory.

In preparation for an observation on drinking, a spider was put into a desiccator at room temperature (*Tarentula barbipes*) or at 35° C. (*Lycosa radiata*). Experience showed that when a spider had lost about 10 % of its normal weight it would nearly always make efforts to drink when given the opportunity. A loss of 20 % was usually fatal and to avoid casualties desiccation was done slowly. In the present series of experiments a spider usually remained in the desiccator for 2 days before use. It was then placed in a small capsule of cigarette paper and weighed on a chemical or torsion balance; given access to the desired moisture conditions; and then weighed again. Defaecation between the two weighings was a very rare occurrence.

---

* Fellow of King's College, Cambridge.

Fig. 1 shows the apparatus used in these experiments, the moisture content of the soil being controlled by the suction* applied to it. The relation between moisture content and suction for any soil was determined as follows. The apparatus was adjusted so that the sintered glass disk was level with the water in the burette and this level was then read. A dried weighed sample of soil was spread over the sintered glass to a few millimetres' depth. The soil absorbed water, and, after readjustment, the burette level was read again and the saturated water content of the soil thus determined. A series of burette readings was then taken at different suctions. As there was a small change of water level with suction in the absence of soil, this was determined separately and a correction made. The percentage moisture content of the soil at each suction was then found.

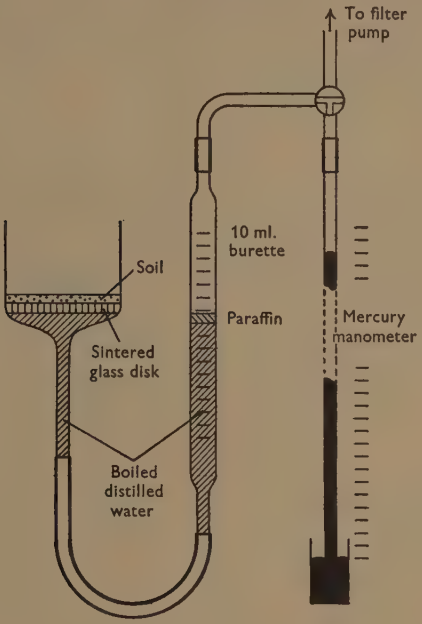

Fig. 1.

In most natural soils the particles, and therefore the capillaries between them, are varied in size. As the suction increases the capillaries empty in order of size, with the result that the moisture content gradually decreases. For the present purpose 'soils' of more uniform particle size were often more informative, besides giving repeatable results, and different grades of carborundum powder have been used, namely grade 100 (mean particle diameter 0·0049 in.), grade 400 (0·0010 in.)

---

* Throughout this paper the word 'suction' is used when referring to the reduced pressure in the soil capillaries, and is measured in mm. Hg below atmospheric pressure. Also the word 'soil' is sometimes used to cover both natural soils and carborundum powders.

and grade 600 (0·0005 in.). The moisture-suction characteristics of these powders are shown in Figs. 2–4, from which it will be seen that the moisture content remains approximately constant except over a critical range of suction, thus indicating the uniformity of capillary size.

In performing an experiment on drinking, the apparatus was first set up with the desired soil and suction. The spider, after the preliminary treatment already described, was put on the soil and if necessary watched under a binocular microscope. If the spider was left unattended, the container was covered with muslin through which there was sufficient ventilation to avoid condensation.

The standard significance tests have been used, namely the *t*-distribution and Fisher's distribution of the variance ratio.

RESULTS

Experiments demonstrating the ability to take water from soil

Table 1 shows the weight increase of desiccated adult *Tarentula barbipes* when put on to a sandy soil at various suctions and left for periods of up to a day. Significant increases occur up to a suction of 90 mm. Hg (to which the increase of 4·9 mg. relates) but not at greater suctions than this.

Table 1. *Weight increase (mg.) of* Tarentula barbipes: *uptake of water from a sandy soil*

(The spiders were all between 31 and 43 mg. in weight, and were left on the soil for varying periods up to 24 hr.)

Suction (mm. Hg)				
25–44	45–64	65–84	85–104	105–119
3·6	0·3	0·0	4·9	0·9
7·9	5·0	2·7		0·5
4·1	5·5	6·1		2·0
5·2		0·0		−1·9
		6·6		
		3·5		
		1·1		
		4·1		
		1·2		

Failure to drink from soil at high suctions may be due to two causes: there may be little water left in the soil, or the spider may be unable to overcome the suction even though plenty of water remains. These two effects are demonstrated separately with the use of carborundum powders. Figs. 2–4 show the weight increases of individual desiccated spiders (*T. barbipes*) when put on to grades 100, 400 and 600 carborundum powders at different suctions. Examination of these results, in conjunction with the moisture characteristics of the powders, suggests (*a*) that failure to drink from grades 100 and 400 above about 80 and 350 mm. respectively, is due to the powders having passed their critical points so that very little moisture is available; and (*b*) that failure to drink from grade 600 above about 450 mm., which is below the critical point of the powder, is due to the spider being unable to overcome the high suction. Evidence will be given below to show that this is not a sudden failure, but that there is a decrease in the rate of drinking with increase of suction.

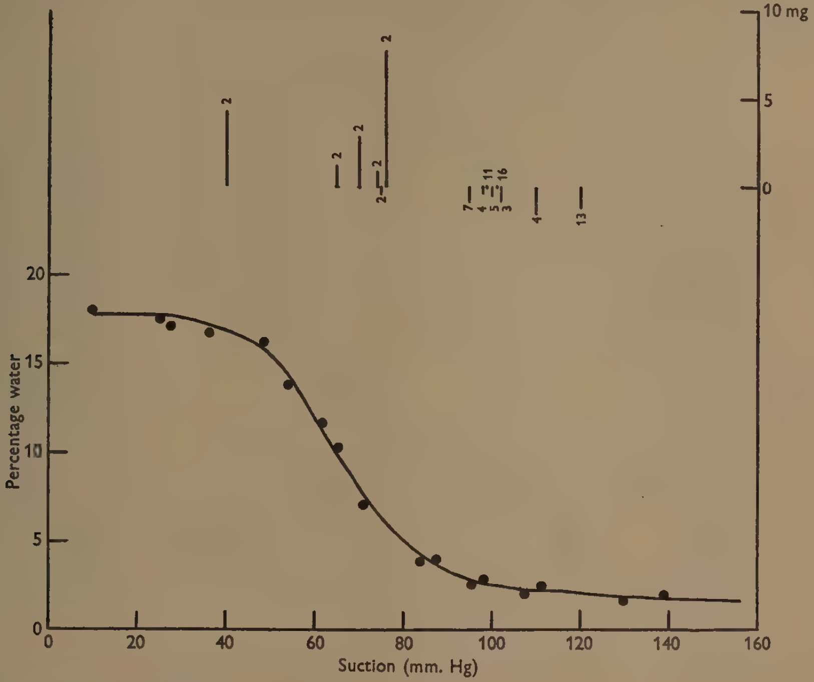

Fig. 2. The moisture-suction characteristic of grade 100 carborundum powder; and the weight increases of individual spiders (*T. barbipes*) after having been left on this powder at different suctions for the stated times (in hours).

Experiments dealing with the rate of drinking

The spiders take up a characteristic attitude while drinking, so enabling the rate of drinking to be determined. The results obtained are summarized in Tables 2–6, to which reference will be made below.

Tables 2*a* and *b* show the uptake of water during two consecutive periods of 20 min. (*T. barbipes*) or 10 min. (*Lycosa radiata*). In neither species is there a significant difference between the uptake during the initial and final periods, suggesting that such factors as fatigue and degree of desiccation do not have an important influence on the rate of drinking over the periods of time considered.

Table 3 (*Tarentula barbipes*) shows no significant difference between the rates of drinking of spiders B and D on grade 400, 150 mm. Hg; or between spiders A and B

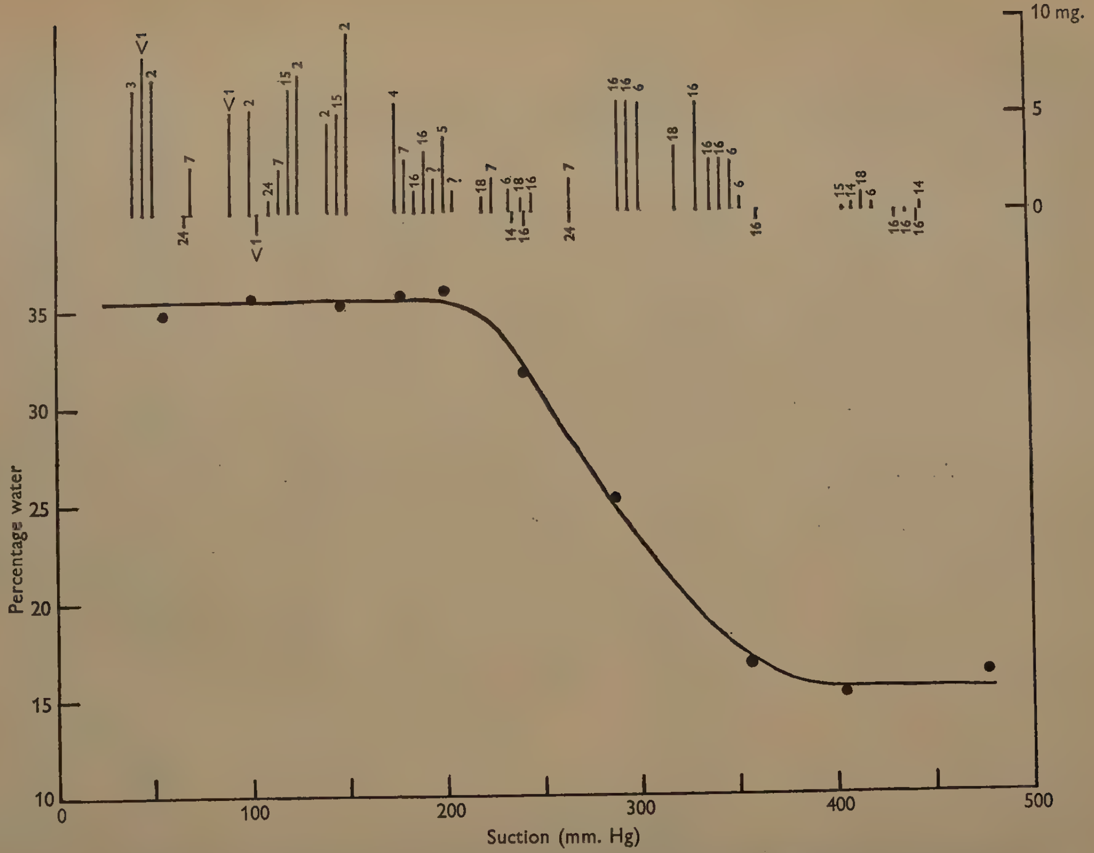

Fig. 3. The moisture-suction characteristic of grade 400 carborundum powder; and the weight increases of individual spiders (T. [illegible]) after having been left on this powder at different suctions for the stated times (in hours).

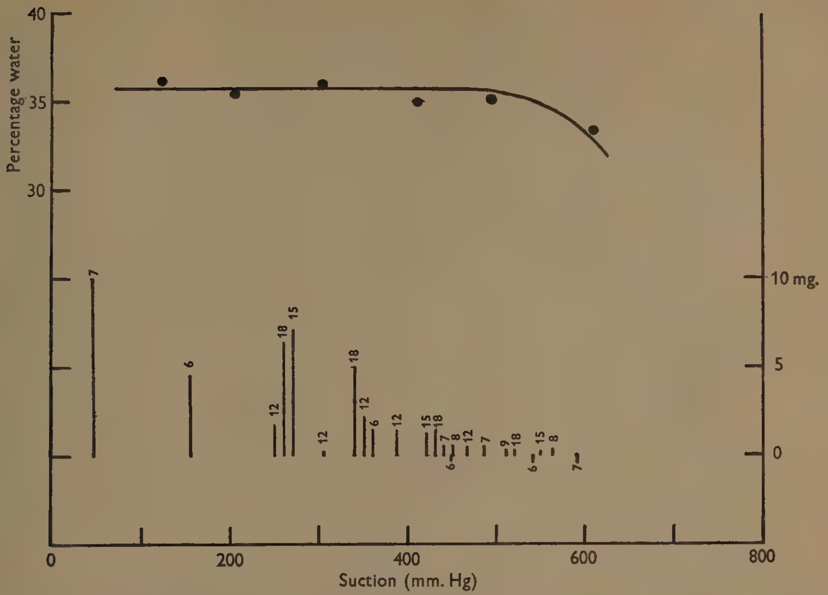

Fig. 4. The moisture-suction characteristic of grade 600 carborundum powder, and the weight increases of individual spiders (*T. barbipes*) after having been left on this powder at different suctions for the stated times (in hours).

Table 2*a*. Tarentula barbipes: *uptake of water by individual spiders during consecutive periods of* 20 *min.*

Grade	400	600	600	600
Suction (mm. Hg) ...	150	150	150	200
1st 20 min. (mg.) 2nd 20 min. (mg.)	2·5 3·0	3·0 4·0	2·5 5·0	1·5 1·5

Table 2*b*. Lycosa radiata: *uptake of water by individual spiders from* 600 *grade carborundum,* 150 *mm. Hg suction, during consecutive periods of* 10 *min.*

1st 10 min. (mg.) 2nd 10 min. (mg.)	14·5 15·7	17·7 12·0	8·2 9·2	12·6* 6·1*	13·5 11·0	16·5 12·5

* Two periods of 7 min. each.

on grade 600, 150 mm. Hg. This indicates the unimportance of unanalysed internal factors. It should, however, be noted that these spiders are of approximately the same size. Some evidence will be given below that the rate of drinking may be affected by size.

The difference in the rate of drinking of *T. barbipes* A, B and D (taken together)

Table 3. Tarentula barbipes: *rate of drinking from different grades of carborundum and at different suctions*

(m = weight increase in mg.; t = time of drinking in min. and sec.; v = rate of drinking in μg./sec.)

Suction	Grade 400									Grade 600								
	Spider A*			Spider B*			Spider D*			Spider A*			Spider B*			Spider D*		
	m	t	v	m	t	v	m	t	v	m	t	v	m	t	v	m	t	v
150 mm. Hg	2·5	17^{48}	2·3	6	28^{0}	3·6	3	30^{0}	1·7	5	16^{0}	5·2	9	30^{0}	5·0	8·5	33^{21}	4·2
				3	19^{3}	2·6	4·5	30^{0}	2·5	6	20^{0}	5·0	7	52^{0}	2·2	4·5	20^{0}	3·8
				3	40^{35}	1·2	8	20^{0}	6·7	4·5	20^{0}	3·8	3	17^{13}	2·9	1·5	12^{34}	2·0
				3	53^{3}	1·0	2·5	20^{0}	2·1	2·5	20^{0}	2·1	3	20^{0}	2·5			
				5	9^{28}	8·8	3	20^{0}	2·5	5	20^{0}	4·2	4	20^{0}	3·3			
				3·5	20^{0}	2·9	1·5	20^{0}	1·3	4·5	20^{0}	3·8	2	14^{13}	2·3			
										3	20^{0}	2·5	3	20^{0}	2·5			
Mean rates†			2·3			2·3			2·7			3·7			3·0			3·7
					2·5									3·4				
200 mm. Hg										4	20^{0}	3·3	2·5	20^{0}	2·1	3	20^{0}	2·5
													1·5	20^{0}	1·3	2	20^{0}	1·7
													1·5	20^{0}	1·3			
Mean rates†												3·3			1·5			2·1
														2·0				

* The sizes of these spiders, expressed as carapace widths, were: A = 3·5 mm., B = 3·0 mm., D = 3·3 mm.

† The mean rates shown in this and subsequent tables have been calculated from the sums of the weight increases and times of drinking, and not from the individual rates of increase.

Table 4. Lycosa radiata: *rate of drinking from different grades of carborundum and at different suctions*

(m=weight increase in mg.; t=time of drinking in min. and sec.; v=rate of drinking in μg./sec.)

Suction	Grade	Spider A*			Spider C*			Spider D*			Spider E*			Spider F*			Spider G*			Mean rates†
		m	t	v	m	t	v	m	t	v	m	t	v	m	t	v	m	t	v	
75 mm. Hg	400	15·4	20^{0}	12·8	15·4	17^{0}	15·0	21·6	16^{10}	22·3	4·8	9^{30}	8·4	11·0 6·9	11^{30} 10^{31}	16·0 11·0				14·8
	600	27·3	20^{0}	22·8	20·0 24·9	20^{0} 20^{0}	16·7 20·8	23·6 22·6	20^{0} 20^{0}	19·7 18·9	8·9	14^{58}	9·9	18·7	17^{50}	17·5	27·3	19^{45}	23·0	18·9
Mean rates†				17·8			17·6			20·1			9·3			15·3			23·0	17·4
150 mm. Hg	400	5·7	20^{0}	4·8	3·2	20^{0}	2·7	3·3	20^{0}	2·8	3·0	20^{0}	2·5	5·9 4·2	20^{0} 20^{0}	4·9 3·5				3·5
	600	2·7	20^{0}	2·3	4·5	20^{0}	3·8	1·6	20^{0}	1·3	3·5	20^{0}	2·9	6·7	20^{0}	5·6	2·5	20^{0}	2·1	3·0
Mean rates †				3·5			3·2			2·0			2·7			4·7			2·1	3·3

* The sizes of these spiders, expressed as carapace widths, were: A=5·5 mm.; C=5·1 mm.; D=5·6 mm.; E=3·7 mm.; F=5·0 mm.; G=5·6 mm.

† See note † at the foot of Table 3.

on grade 400, 150 mm. Hg, is not significantly different from the rates of these spiders on grade 600 at the same suction (Table 3). Similarly, there is no significant difference between the rates of drinking of the individuals of *Lycosa radiata* on 400-grade and 600-grade at either 75 mm. or 150 mm. (Table 4).

No *L. radiata* ever drank at a suction of 200 mm. Hg or over (seven observations). At 150 mm. they drank at one-fifth the rate at 75 mm. (Table 4); while the rate of drinking from a free water surface or saturated filter-paper (Table 6) was over 10 times the rate at 75 mm. Similarly, the rate of drinking of *Tarentula barbipes* from free water (Table 5) was about 10 times the rate at 150 mm.; and the small number of observations (Table 3) made at 200 mm. (which is well below the limiting suction for this species—see Fig. 4) show the rates to be significantly less than at 150 mm.

The rates of drinking of free water by both *T. barbipes* and *Lycosa radiata* (Tables 5 and 6) are significantly correlated with size. The spiders used for the suction experiments were not sufficiently varied in size for this effect, if present, to show itself (although *L. radiata* 'E' of Table 4 is smaller than the rest, and its rate of drinking at 75 mm., but not at 150 mm., is lower than the others).

Table 5. Tarentula barbipes: *rate of drinking from a free surface by spiders of different size*

Carapace width (mm.)	Rate (μg./sec.)					Means*
4·0	37·3	58·2	50·0	53·3	—	49·7
3·5	50·7	48·3	39·3	50·1	45·8	46·8
2·8	37·5	30·8	25·0	44·4	30·7	33·7
2·5	22·0	17·1	—	—	—	21·4
2·5	25·0	—	—	—	—	

* See note † at the foot of Table 3.

DISCUSSION AND CONCLUSIONS

It has been shown that certain lycosid spiders can drink the moisture in soil and carborundum powders, the rate of drinking depending on the suction to which the capillary water is subject. With increasing suction, drinking becomes impossible from any particular powder, either when most of the capillaries empty, or when the suction exceeds a certain value, whichever happens first. The limiting suction, beyond which drinking is impossible even from a saturated powder, depends on the species of spider and perhaps also on the size of the individual. These results are not incompatible with the idea that for the extraction of soil capillary water a spider is dependent upon the power of its pumping stomach. This aspect of the subject will be dealt with elsewhere when the structure of the foregut is considered.

No information has yet been obtained concerning the importance of soil moisture in the natural life of spiders, and no doubt it varies with species, environment and season. For instance at the time of year when the specimens of *L. radiata* were collected for this work, the soil of the stream-bed in which they were mostly found was dry and powdery; but earlier in the summer there must have been a period after the disappearance of the running water when the soil remained moist, and this moisture may then have been used. In general, no one who has watched a desiccated spider immediately start drinking when put into moist soil, or excitedly explore the surface when the suction is too high, is likely to believe that the animal had never so acted before in its individual or racial history.

Table 6. Lycosa radiata: *rates of drinking from a free surface, grouped according to size of spider (carapace width in mm.). The horizontal lines across the columns separate the results from different spiders*

Size group ...	3·5–4·4	4·5–5·4	5·5–6·4	6·5–7·4	7·5–8·4
Rate (μg./sec.)	107 114 128 130 80 53	344 241 198	207 289	329 350	562 539 209 247
	83 174 138 133 111	273 215 209	216		470
	127 105 126		288 197 170 201		
			275 268 337		
			244 299 334 318		
Means*	104	232	250	336	349

* See note † at the foot of Table 3.

SUMMARY

1. A study has been made of the drinking of soil moisture by two species of lycosid spider. Carborundum powders have been used to provide a 'soil' of uniform particle size.

2. The rate of drinking falls off with increase in the suction applied to the capillary water, finally becoming negligible, even though the soil remains saturated, at a suction which varies with the species of spider and possibly with the size of the individual.

3. This limiting suction is never reached if the capillaries empty at a lower suction.

4. The rate of drinking does not fall off with time, is similar for different individuals of the same species and size, and is not affected by soil particle size at a given suction, provided this is below the critical suction for the soil.

I am grateful to Dr N. Collis-George of the School of Agriculture, Cambridge, for introducing me to the method of soil-moisture determination. The observations on *L. radiata* were made at the Laboratoire Arago, Banyuls-sur-Mer, France, which I visited with the help of a grant from the research fund of King's College, and where I received every help and kindness from Prof. G. Petit and his staff.

THE OXYGEN UPTAKE OF THE LOBSTER (*HOMARUS VULGARIS* EDW.)

By H. J. THOMAS

Scottish Home Department, Marine Laboratory, Aberdeen

(*Received* 1 *May* 1953)

I. INTRODUCTION

Lobsters are abundant on the grounds off the west coast of Scotland and the Outer Isles, but a full exploitation of these stocks is, in part, prevented by the difficulties of marketing. Ice is not readily available and mortality in transit can, in summer, often account for the greater proportion of a consignment. Furthermore, as stated in *Practical Hints for Lobster Fishermen* (Anon. 1952), the selling price for lobsters is at its lowest in summer, and storing lobsters caught during the summer for sale in the winter market with its higher prices is well worth while. In view of the economic importance of the lobster fishery to the crofting counties of Scotland, a knowledge of the fundamental requirements for satisfactory storage and transit is desirable. Having regard to the need for such data, the present research into the oxygen requirements of the lobster was carried out, on behalf of the Development Commissioners, at the Marine Laboratory of the Scottish Home Department, Aberdeen.

Research into the lethal temperatures, salinities and oxygen concentrations in *Homarus americanus* is at present being conducted by Wilder and others of the Fishery Research Board of Canada. In addition, a considerable amount of work has previously been done on the oxygen requirements of a variety of invertebrates, with special reference to their respiratory quotients and metabolism. A summary of this, with a bibliography, is given by Prosser (1950). In the case of the lobster the previous figures quoted for oxygen consumption are very variable. Rogers (1938) gives the oxygen uptake at 15° C. in *Homarus* (species unquoted) of weight 315 g. as 0·0972 g./kg./hr. This is equivalent to 0·069 ml./hr./g. body weight. Amberson, Mayerson & Scott (1924) showed that the oxygen consumption of *H. americanus* varied with oxygen tension, and plotted the total oxygen uptake for one animal at 20° C. They do not give data for other temperatures, nor is the weight of the animal quoted. It is, therefore, impossible to convert their data into the more usual ml./hr./g. body weight for comparison with other results. Weymouth, Crismon, Hall, Belding & Field (1944) have shown that in the case of the kelp crab. *Pugettia producta*, the oxygen consumption per gram body weight decreases with increasing size of animal, and this relationship appears to be of general application throughout the animal kingdom, both interspecifically and intraspecifically (Zeuthen, 1953). The oxygen uptake of *Homarus americanus* as found by Bosworth, O'Brien & Amberson (1936) is quoted in Table 1.

The data in Table 1 are incomplete and in many ways conflicting. The present work is an attempt to reconcile these results and to evaluate the effect of the more important variables, the interplay of which fixes the oxygen uptake in the lobster, *H. vulgaris*, under a particular set of circumstances.

Table 1. *Oxygen consumption in* Homarus americanus, *ml./hr./g. body weight*

(From Bosworth, *et al.* 1936.)

Temperature (° C.)	15	15	15	15	22	22
Weight (g.)	189	189	189	189	324	230
Oxygen concentration (ml./l.)	1·39	2·28	2·35	3·25	3·45–0·80	4·97–0·84
Oxygen consumption (ml./hr./g.)	0·021	0·020	0·025	0·035	0·039	0·037

II. MATERIALS AND METHODS

(*a*) *Materials*

Lobsters of both sexes, of weights ranging from 300 to 800 g., were used in the experiments. Females carrying spawn, soft lobsters or individuals showing signs of relatively recent moulting were not used. The material is therefore wholly within the legal marketable range of 9 in. and over. Specimens were obtained in small numbers from the Berwickshire coast in February and March 1952, and from the Orkney Islands monthly from May 1952 to March 1953.

(*b*) *General procedures*

On receipt, lobsters were transferred to large aquarium tanks, maintained at room temperature; they were not fed. Experiments were commenced after the lobsters had been retained 2 days in the tanks. Brunow (1911) records that in the crayfish, *Astacus fluviatilis*, oxygen uptake decreases with protracted starvation (up to 20 weeks). In the present work on lobsters the maximum period of starvation was 4 weeks, over which interval no variation in oxygen uptake was noticeable.

Prior to carrying out an experiment the lobster was weighed and, where appropriate, the volume was taken. It was then kept overnight in sea water, the temperature of which was controlled to the initial experimental temperature. A standard drainage time of 3 min. was allowed prior to weighing, and a similar procedure followed before measuring the volume by displacement. Only perfect specimens of lobsters were used, and at the conclusion of the final experiment the gills were examined to establish freedom from *Nicothoë astaci*. This parasite was prevalent in Scottish waters during 1952. In transferring lobsters between tanks, and in particular to the experimental tank, the specimen was slowly lowered into the water, tail first. If this precaution is not taken there is a risk of air becoming locked in the gill-chamber, resulting in anomalous data.

All toxic materials were avoided. Glass aquarium tanks were used, and stirrers, thermostats, cooling coils and heaters were of glass or stainless steel.

(*c*) *The method A*

One method used for the determination of oxygen uptake is similar to that used by Amberson *et al.* (1924). In the present case the lobster was confined to an area 10 × 6 in. at one end of a glass aquarium tank by a transverse partition of glass rods spaced 1 in. apart. This serves to reduce the movement and achieves more nearly basal conditions. The initial volume of sea water in the tank, which was thermostatically controlled and stirred throughout the experiment, was 20 l. The water was covered by a layer of medicinal paraffin of about 1·5 cm. thickness. Duplicate samples of the sea water of accurately known volume, about 200 ml., were withdrawn from the tank by siphon at stated intervals. The amount of dissolved oxygen in samples was determined using Jacobsen's (1921) modification of the Winkler (1888) method. Initial and final pH determinations were made colorimetrically. The accumulation of nitrites was determined by the method of Orr (1926). This procedure will be referred to as method A.

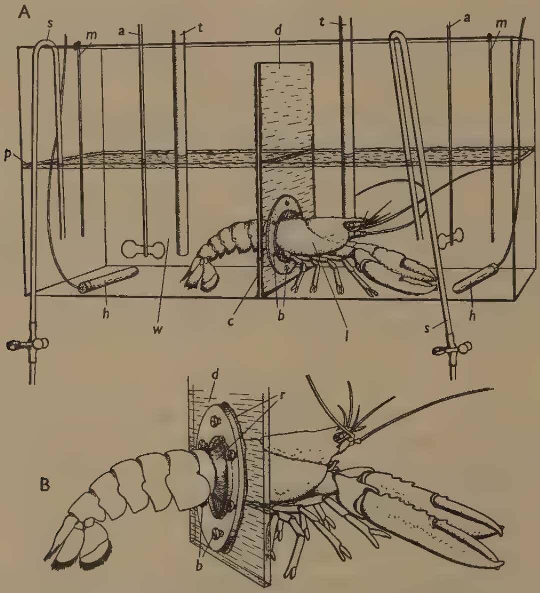

Fig. 1. The apparatus used to determine the oxygen uptake by the gills and the abdomen respectively: A, the complete apparatus; B, the method of holding the lobster in the partition. *a*, stirrer; *b*, bolts; *c*, cement; *d*, dividing partition; *h*, heater; *l*, lobster; *m*, thermometer; *p*, paraffin layer; *r*, rubber disk; *s*, siphon sampling tube; *t*, thermostat; *w*, sea water.

Observations on the behaviour of the lobster (referred to later, p. 239) suggest that, in addition to oxygen uptake via the gills, the abdominal swimmerets subserve a respiratory function. This hypothesis was tested in experiments using the modified method A apparatus shown in Fig. 1. Female lobsters only were used. The specimen was held at the first abdominal segment in a shaped hole in a rubber disk. In this way the oxygen uptake due to the cephalothorax is confined to one section of the tank whilst the oxygen uptake of the tail runs down the oxygen concentration of the sea water in the other section. Twelve litres of sea water were used initially in each section. To avoid pressure differences, the partition was positioned so that withdrawal of samples maintained the same levels of sea water in both parts. Simultaneous control experiments were carried out to measure the variation in oxygen concentration in sea water with no lobster present.

(*d*) *The method B*

Keys (1930) has discussed the methods employed generally in the determination of the gas exchanges of aquatic animals. In addition, Allee & Oesting (1934) have shown the limitations of Winkler's method for determining oxygen uptake in experiments similar to method A due to the possible accumulation of nitrites. Apart from inaccuracies in the oxygen analysis, the possibility of accumulated excretory products having a physiological effect on the animal cannot be excluded. Furthermore, the method A type of experiment can only be carried out daily at one control temperature. To determine the effect of temperature change, the individual has to be subjected to a protracted series of experiments. Although this was, in fact, done on succeeding days, it nevertheless constitutes a considerable limitation. A method was devised to overcome these objections as well as to give additional information. The set-up in this type of experiment, method B, is shown in Fig. 2.

A cylindrical, celluloid cowl of overall length 11–14 cm., of the shape shown in Fig. 2B, was constructed with an internal diameter such that it gave a loose-touching fit when placed over the anterior half of the cephalothroax of the lobster. The cowl was cemented into position (Fig. 2C) by gelatine melting at 30–35° C. Before fitting, a film of gelatine was painted on to the back and sides of the cephalothorax, and on to the inside of the celluloid cowl. The projecting sides of the fitted cowl ran about 0·5 cm. above the edge of the cephalothorax and the ventral part of the cowl lay immediately in front of the bases of the big claws. The whole of the edge of the cowl was affixed with gelatine which overlapped on to the cephalothorax. Care was exercised to avoid running gelatine over the setae at the edge of the cepahlothorax, thereby interfering with the inflow of water into the gill chambers. Gelatine was run in ventrally between the lobster and the cowl in front of the great claws. A complete seal was thus made, shutting off the exhalant water from the gill chambers which was thereby collected within the cowl. Since the edge of the cephalothorax in the region of the big claws is a dead area from the point of view of gill ventilation, it was possible to seal the cowl into position without affecting the flow of water used for respiration. A smearing of petroleum jelly was made on the dorsal and lateral sides over the gelatine in order to protect it from solution. The cowl should be correctly fitted. If too large, it tends to soak off, whilst if too small, it presses upon the sides of the cephalothorax thereby unnaturally reducing the rate of flow.

The cowl having been cemented into position, the lobster was lowered gently, tail first, into the experimental tank and a rubber tube, filled with water, was fixed into position as shown in Fig. 2. Rubber tubing of internal diameter 0·7 cm. was deemed to have negligible resistance to the flow even at the highest rate of pumping investigated. Tubing of this diameter was used except where restriction of the flow was required. Between samplings, the level of the outlet end of the rubber tubing was maintained at the same level as the water in the tank, thus avoiding any siphoning effect. Under these circumstances, the water coming over was wholly due to the respiratory pumping action of the animal. Samples of exhalant water were collected in glass-stoppered bottles of accurately known volume. In sampling, the level in the bottle was maintained at exactly the same level as the water in the experimental tank. The time taken for filling the bottle was recorded, thus giving a measure of the rate of ventilation. The water in the experimental tank was thermostatically controlled and stirred. The lobster was retained in a small section at one end of the experimental

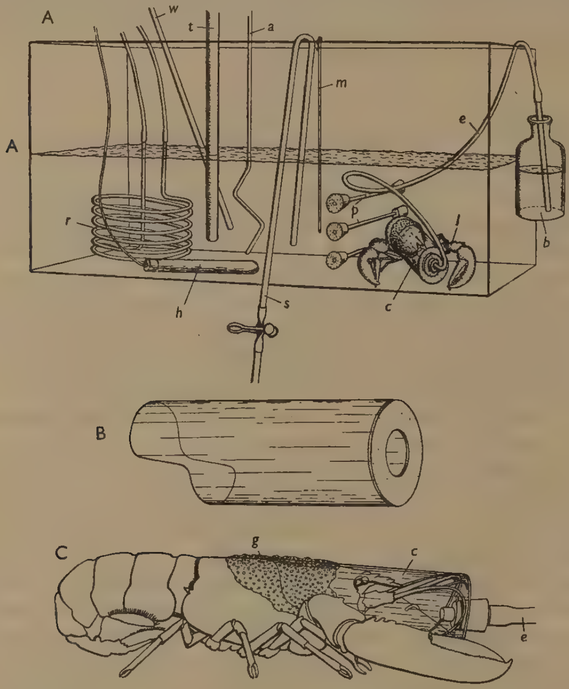

Fig. 2. The apparatus used, in the method B, to determine the oxygen uptake by the gills. A, the complete apparatus; B, the cowl; C, the method of fixing the cowl. *a*, Stirrer; *b*, sample bottle collecting exhalant water; *c*, cowl; *e*, exhalant tube; *g*, gelatine cement; *h*, heater; *l*, lobster; *m*, thermometer; *p*, partition of glass rods; *r*, cooling coil; *s*, siphon tube for sampling tank water; *t*, thermostat; *w*, replacement sea water supply.

tank, thereby maintaining more nearly basal conditions. The presence of the cowl, by hampering movement, had a similar effect. Samples of the tank water were drawn off by siphon. The experiment having been set up, the lobster was left to 'acclimatize' for a minimum of 30 min. before experimental observations were taken. The complete apparatus is shown in Fig. 2A.

In conjunction with the method B described above, the oxygen concentration of the tank water was varied by blowing off the dissolved oxygen with a stream of nitrogen. The effect of carbon dioxide was investigated by adding quantities of sea water saturated with this gas to the water in the experimental tank. Edwards & Irving (1943) record that in the sand crab, *Emerita talpoida*, changes in temperature of 10° C. or less from the normal brought about rapid changes in oxygen consumption. The oxygen consumption rapidly returned to normal when the animals were restored to the original temperature. In the lobster, similarly, it was found that the animal rapidly adjusted itself to changes of temperature of the order 3° C., particularly at temperatures above 10° C. After an interval of less than 10 min. from the time of temperature change, the pumping rate was found to have reached a new constant level. In studying the effect of temperature upon oxygen uptake, the interval between sampling was never less than the period required to attain uniform rate of pumping, and the tank water was maintained constant by thermostatic control at each observation temperature. The rate of ventilation of the gills was large compared with the volume of water contained in the cowl which at the maximum was 120 ml. A minimum volume of 1000 ml. was passed between readings thereby allowing the water in the cowl to be carried off.

The effect of temperature over the range 5–20° C. was investigated, and similar experiments carried out with the flow restricted by using rubber tubing 0·4 cm. internal diameter. An accelerated flow of water over the gills was produced by lowering the output end of the rubber tubing to a measured distance below the surface level of water in the tank. In this experiment samples were collected with an equal degree of siphoning. Where appropriate, such as in the case of varied oxygen concentration and temperature, reverse experiments were carried out; for instance, temperature rising and, conversely, temperature falling.

(*e*) *The respirometer*

Dixon (1943) has reviewed the types of manometer apparatus used in the investigation of respiratory exchanges. A manometric method was adopted to determine the oxygen uptake of the lobster in air. Having been weighed and its volume taken, the lobster was kept overnight previous to the experiment in aerated sea water controlled at the experimental temperature. Before being transferred to the respirometer, the animal was maintained in aerated water at the exact experimental temperature for 10 min. It was transferred immediately to the air-chamber, drainage from the gills occurring in the chamber. Carbon dioxide was absorbed continuously by means of soda lime. The animal was therefore maintained throughout the experiment in a carbon dioxide free atmosphere over sea water. The volume of drainage water was measured at the conclusion of the experiment. The respiration chamber was immersed throughout the period of observation in a constant-temperature bath. Using this technique, variation in oxygen uptake was studied over the temperature range 6–18° C. at temperature intervals of 1·5° C. Each experiment was of about 5 hr. duration and the same lobster was used on succeeding days so as to cover the whole temperature range. A series of experiments was carried out with the temperature being raised, and a similar series with the temperature being reduced.

(*f*) *Treatment of results*

Amberson *et al.* (1924), in their work on the oxygen consumption of *Homarus americanus* at 20° C. by the method A, plotted the oxygen concentration of the tank water against time. The resulting curve proved to be exponential. In this instance, log oxygen concentration plotted against time gave a straight line. The sampling volumes used were about 35 ml. They do not state the initial volume of sea water, but express the opinion that the loss in volume due to sampling does not seriously upset the result. In certain cases the water withdrawn in sampling was replaced by a like amount of water made up to an oxygen tension identical with that indicated by the preceding analysis. In the present experiments it was preferred, in order to increase accuracy, to take duplicate samples each of a larger volume. Together with washings, about 400 ml. were withdrawn at each observation. It was not found practicable to add sea water of reduced oxygen concentration to make good the volume withdrawn. In plotting the oxygen concentration of the tank water against time, a proportionate adjustment has been made in the time scale to compensate for the difference in volume applicable to each observation. A graph on this basis, similar to that depicted by Amberson *et al.*, is shown in Fig. 3. The data can also be used to calculate oxygen uptake based on the decrease in oxygen concentration between readings and the corresponding volume of the sea water in the tank. The amount of oxygen consumed, calculated in this way, is referred to the mean oxygen concentration during the period. Fig. 4 shows the same data as depicted in Fig. 3 presented in this way.

In this instance, as found by Amberson *et al.*, the oxygen uptake is directly proportional to oxygen tension, but the line depicting the relationship (Fig. 4) does not go through the origin, although nearly so. Unless the relationship between oxygen tension and oxygen uptake results in a straight line passing through the origin, the plot of log oxygen concentration against time, as made by Amberson *et al.*, will not be a straight line. Thus, in Fig. 3, the best fit for log oxygen concentration against time would be a slight curve. At higher temperatures the difference is insignificant, but it assumes considerable importance at lower temperatures. For this reason, in the great majority of cases, the calculation of the actual oxygen uptake between readings has been made and the presentation of data, as in Fig. 4, has been preferred. The oxygen uptake throughout has been expressed in terms of ml. of oxygen at N.T.P. per hour per gram of body weight.

In experiments by the method B oxygen uptake has been calculated from the observations of the oxygen concentration of the tank water, oxygen concentration of the exhalant water, and the ventilation rate of the gills.

The treatment of data in the case of experiments on the oxygen uptake in air was in accordance with the procedure given by Dixon (1943). The results, as with other experiments, are expressed as ml. of oxygen at N.T.P. per hour per gram of body weight.

Statistical analyses have been carried out to determine the lines of best fit for the data relating oxygen uptake to oxygen concentration and to temperature. The respective levels of significance are quoted in the figure legends.

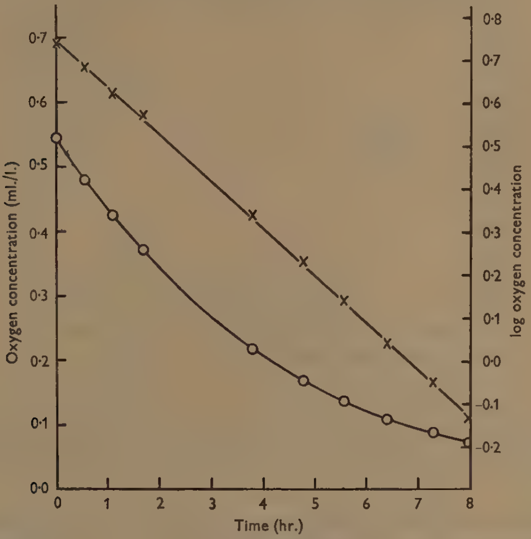

Fig. 3. The decrease, at 15° C., in the oxygen concentration of the medium with time by a male lobster of weight 352 g.; *o*, oxygen concentration; *x*, log oxygen concentration.

III. RESULTS

(*a*) *Total oxygen uptake in sea water*

(i) *The effect of oxygen concentration*

Fig. 4 shows the relationship between oxygen uptake and oxygen concentration at 15° C. for a male lobster of weight 352 g., captured in February 1952. The points of this graph lie close to a straight line significant at the level $P < 0{\cdot}001$. This result is in agreement with the work on *H. americanus* by Amberson *et al.* (1924). The pH at the commencement of this experiment, by the method B, was 8·3, and the final pH was 7·6. Oxygen uptake in this instance is directly proportional to oxygen concentration. The concentration of nitrite at the end of the experiment was equivalent to 0·1 mg. nitrite nitrogen per litre.

Table 2 shows the number of experiments in which oxygen uptake is significantly proportional to oxygen concentration in the case of five lobsters, each of which were investigated over the temperature range 6–18° C. at intervals of 1·5° C.

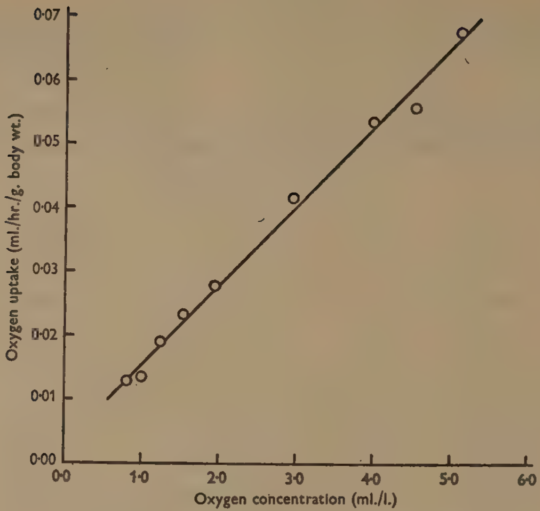

Fig. 4. The effect of oxygen concentration on oxygen uptake at 15° C. From the same data as Fig. 3. ($P<0{\cdot}001$.)

Table 2. *The levels of significance of the straight line relating oxygen uptake to oxygen concentration at stated temperatures between* 6–18° C. *in five female lobsters*

Lobster no.	Weight (g.)	6·0°	7·5°	9·0°	10·5°	12·0°	13·5°	15·0°	16·5°	18·0°	Total significant
27	558	0·05	0·05	0·05	0·01	0·01	0·01	0·001	0·001	0·01	9
28	483	0·001	0·001	0·001	0·01	0·001	0·001	0·001	0·001	0·01	9
31	502	0·01	0·01	0·05	N.S.	N.S.	0·01	0·001	0·05	0·01	7
20	480	0·05	0·05	N.S.	N.S.	N.S.	0·01	0·001	0·001	0·001	6
17	528	0·001	N.S.	0·05	0·05	N.S.	N.S.	0·01	0·01	0·01	6

Of the total forty-five experiments ,thirty-seven showed the oxygen uptake to be directly proportional to the oxygen concentration with a significance of $P<0{\cdot}05$. It can reasonably be concluded that over the temperature range 6–18° C., oxygen uptake is directly proportional to the oxygen concentration of the medium.

(ii) *The effect of sex and size of lobster*

Fig. 5 shows the oxygen uptake of seven female lobsters and five male lobsters of varying weights at 15° C. and at oxygen concentration 5 ml./l. as determined by the method A. These lobsters were all captured in February and March 1952. Weymouth *et al.* (1944) have shown that in *Pugettia producta* there is no significant difference in oxygen consumption between the sexes, and that the relative oxygen consumption decreases at a constant rate with increase of relative body weight, so that on logarithmic co-ordinates the regression of oxygen consumption is a straight line with a positive slope. It seems likely that both these findings hold good for *Homarus vulgaris*. Over the range of weights investigated (300–800 g., covering the great majority of lobsters marketed) there is no apparent difference in oxygen uptake between the sexes and oxygen consumption per gram of body weight is inversely proportional to the weight of the animal (Fig. 5).

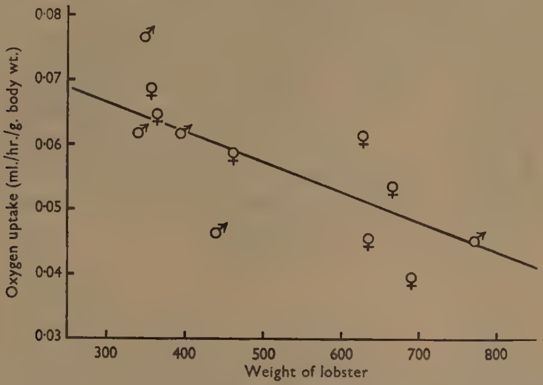

Fig. 5. The oxygen uptake in relation to weight of lobster, males and females.

(iii) *The effect of temperature at a variety of oxygen concentrations*

The general relationship between oxygen consumption and temperature has been surveyed by Krogh (1916), and work on this relationship in a wide variety of poikilothermic animals has been reported by numerous authors. Fox (1936) and Edwards & Irving (1943) have shown that the response to temperature in certain animals may vary to some extent at different latitudes and seasons. The latter authors also have shown in the case of *Emerita talpoida* that the increase in oxygen consumption per unit of weight caused by temperature change tended to be greater in the smaller animals.

As was seen above (Table 2) lobsters nos. 27 and 28, in experiments, by the method A, over the temperature range 6–18° C. showed an oxygen uptake directly proportional to oxygen concentration. The lobsters were females, of weights 558 and 483 g. respectively, caught in Orkney waters of mean surface sea water temperature about 11·7° C. in September 1952. The two sets of data have been combined and the results are shown in Fig. 6. It will be observed, particularly at the lower temperatures, that the lines of best fit relating oxygen uptake and oxygen concentration do not necessarily pass through the origin. The significance of this in the method for treatment of results was discussed in § 2(*f*), p. 234.

Fig. 7 indicates the relationship between oxygen uptake and temperature in sea water at oxygen concentrations 2, 3, 4 and 5 ml./l. respectively, the data having been derived from Fig. 6. At any given oxygen concentration, oxygen uptake increases with increasing temperature.

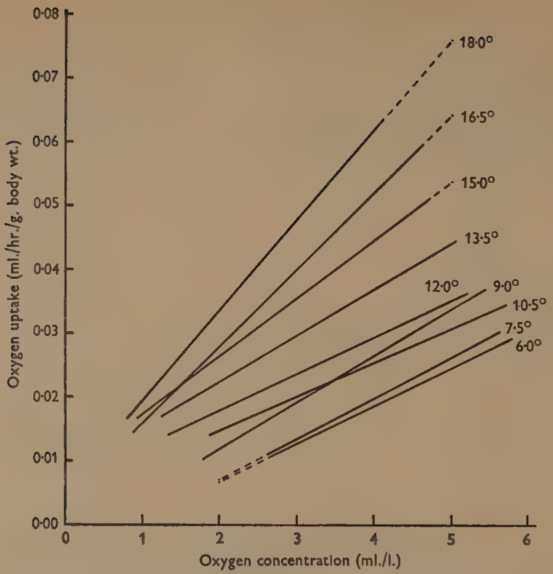

Fig. 6. The oxygen uptake (mean of data for two lobsters, weights 558 and 483 g. respectively) at varying oxygen concentrations for a series of temperatures.

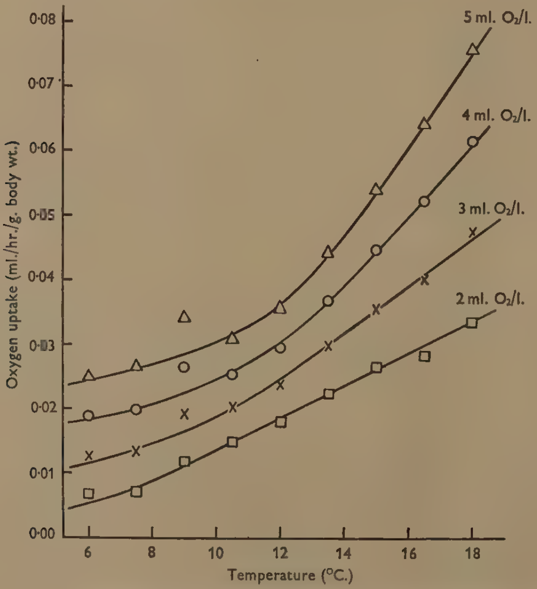

Fig. 7. The effect of temperature on oxygen uptake at a series of oxygen concentrations. From the same data as Fig. 6.

(iv) *The respiratory areas*

During the later stages of experiments by the method A, lobsters were observed to hold the abdomen in a high, open arch, with the abdominal swimmerets pointing to the centre. When returning lobsters to aerated water at the conclusion of an experiment, they generally paddled strongly with the abdominal swimmerets and moved around the tank. A less common, but not infrequent, behaviour was for the swimmerets to beat vigorously, their tips coming together roughly midway along the length of the abdomen, and then moving outwards. There was no progression of the animal with this type of activity. The reasonable interpretation is that the action serves to ventilate the abdominal swimmerets and that these subserve a respiratory function.

This hypothesis was investigated by a modification of the method A whereby the tail and head components were separated (Fig. 1); the procedure has been described in § 2(*c*), p. 230.

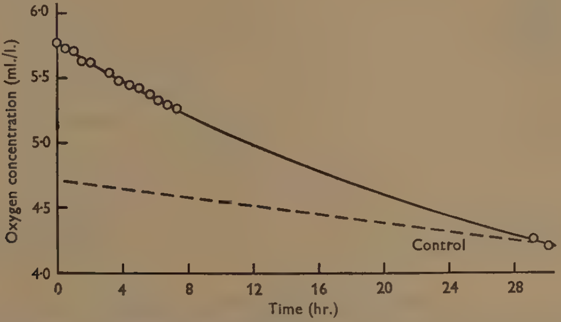

Fig. 8. The decrease in the oxygen concentration of the medium with time, brought about by the abdomen of lobster no. 26, weight 504 g. Temperature, 15° C.; the supply of sea-water to the gills was continually aerated.

Fig. 8 shows the decrease in the oxygen concentration of the medium at 15° C. by the abdomen of a female lobster of weight 504 g., caught in September 1952. In this experiment the section of the tank housing the anterior part of the animal was liberally aerated. Compared with the control, which consisted of a similar volume of sea water overlaid by paraffin and maintained at 15° C., the tail decreased the oxygen concentration of the water.

Two further experiments were carried out also at 15° C. on the same lobster. In these the cephalothorax was allowed to decrease the oxygen concentration of the sea water in its compartment whilst the tail decreased the concentration in the other compartment. Fig. 9 shows the decrease in oxygen concentration in the tail compartment with time in each of these experiments as well as the corresponding decrease for the experiment in which the cephalothorax compartment was aerated. The initial oxygen concentrations varied slightly in these experiments. In the two cases where the gills were allowed to decrease the oxygen in their compartment, the fall in oxygen concentration in the abdomen compartment was equivalent to 1·04 and 1·10 ml./hr. respectively. The corresponding depletion of oxygen in the experiment where the water in the gill section of the tank was aerated was at the lower rate of 0·83 ml./hr. It is reasonable to attribute the difference to an internal physiological effect. The oxygen want of the animal is likely to be less where the water ventilating the gills is adequately aerated.

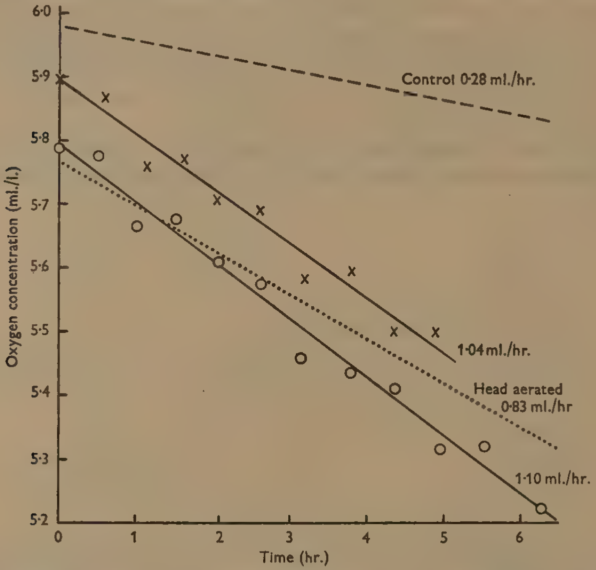

Fig. 9. The decrease, with time, in the oxygen concentration of the medium in two experiments at 15° C. by the abdomen of lobster no. 26. Simultaneously the cephalothorax decreased the oxygen concentration of the sea water in the anterior compartment (Fig. 10). The corresponding depletion of oxygen in a control and the rate of depletion in a similar experiment in which the anterior compartment was aerated (Fig. 8) are also shown.

Fig. 10 shows the variation in oxygen uptake of the gills only with varying oxygen concentration. The results were obtained from the experiments, by the method A, referred to above, the corresponding data for the tail sections being given in Fig. 9. Taking the mean decrease in oxygen concentration in the tail compartment in these two experiments and making an allowance for the decreasing oxygen concentration in the control experiment, the oxygen uptake by the abdomen at an oxygen concentration of 5·3 ml./l. is approximately 3 % of the total oxygen uptake of the animal, being the sum of the oxygen uptakes of the abdomen and the cephalothorax. On this basis of a 3 % uptake by the abdomen, a second scale is shown in Fig. 10 indicating the total oxygen uptake expressed as ml./hr./g. body weight. Brunow (1911) found the gill area in *Astacus fluviatilis* to be 3·035 cm.^{2}/g. body weight. Rogers (1938) quotes the average area of gill surface in *Carcinus maenas* as 7·7 cm.^{2}

and in *Cancer pagurus* as 7·8 cm.^{2}/g. body weight. No data could be found regarding the gill area in *Homarus*. In view of the structure and behaviour of the animal it is reasonable to attribute oxygen uptake by the abdomen primarily to the swimmerets. Having regard to the area of the swimmerets, the level of oxygen uptake by the latter at 3 % of total uptake appears reasonable.

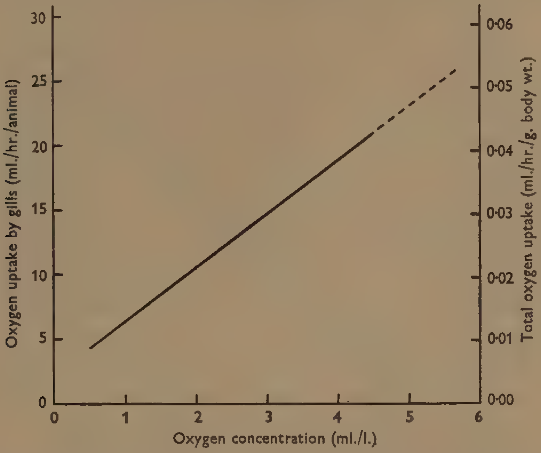

Fig. 10. The oxygen uptake, by the gills alone, in relation to oxygen concentration for two experiments at 15° C., and the total oxygen uptake by the same lobster, no 26. Refer to Fig. 9. ($P < 0·001$.)

(*b*) *Oxygen uptake by the gills in sea water*

(i) *The effect of oxygen concentration*

The oxygen concentration of the sea water in the experimental tank was varied by blowing off the oxygen with a stream of nitrogen. The latter procedure was found not to vary the pH significantly as compared with well-aerated sea water as at the initial sampling in each experiment. Five experiments were carried out in which a lobster was subjected to a varying oxygen concentration by this means and the corresponding oxygen uptake determined using method B.

Fig. 11 shows the variation at 15° C. in oxygen uptake by the gills with oxygen concentration in a male lobster of weight 322 g., captured during March 1953. The percentage of oxygen extraction is also shown. As with experiments by the method A, the oxygen uptake directly proportional to oxygen concentration. In the method B, as opposed to the method A, there is no accumulation of excretory products and the pH is almost unchanged.

The relationship between oxygen concentration, rate of ventilation, percentage extraction and oxygen uptake by the gills in another experiment at 15° C., on a female lobster of weight 385 g., caught in February 1953, is shown in Table 3. The rate of ventilation of the gills was independent of the oxygen concentration of the medium.

Five such experiments were carried out, and in no case did the results show an accelerated rate of ventilation of the gills with decreased oxygen concentration.

The pulsation of the exopodite of the first maxilliped was recorded at varying oxygen concentrations in an experiment in which the oxygen concentration of the tank water was reduced by nitrogen. The results showed no positive response to decreasing oxygen concentration in the movement of the respiratory appendages. As in the case of *Astacus fluviatilis* (Fox & Johnson, 1934), when the animal moved its body and limbs, the rate of beat of the respiratory appendages immediately rose. Thereafter the rate slowed down and returned to its initial value in 7–10 min., irrespective of the oxygen concentration of the medium. This result is in agreement with the rates observed in experiments, by the method A, for ventilation of the gills. It conflicts, however, with Prosser (1950), who records a positive response of the

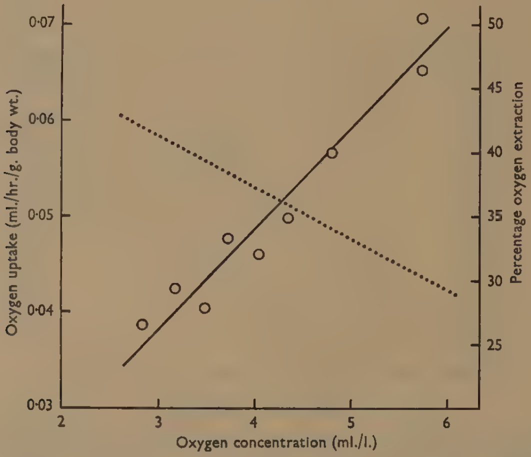

Fig. 11. The effect of oxygen concentration of the medium on the oxygen uptake and the percentage of oxygen extracted by the gills of a lobster, weight 322 g.; continuous line depicts oxygen uptake ($P < 0{\cdot}001$); dotted line depicts percentage of oxygen extraction ($P < 0{\cdot}05$).

Table 3. *The relationship between oxygen concentration, rate of ventilation, percentage extraction and oxygen uptake at* 15° C.

Oxygen concentration (ml./l.)	Rate of ventilation (l./hr.)	Percentage extraction	Oxygen uptake (ml./hr./g. body weight)
5·78	9·83	30·6	0·045
5·78	9·72	31·4	0·046
4·63	9·22	36·6	0·041
3·77	9·51	42·1	0·039
3·23	9·52	47·9	0·038
2·75	9·87	54·1	0·038
2·44	9·56	55·1	0·033

scaphognathite in *Homarus vulgaris*, both to oxygen want and to increase of carbon dioxide. This subject is referred to again in § 4, p. 248.

Whilst in the experiments by the method B the rate of ventilation of the gills was unaffected by the oxygen concentration of the medium, the animal responded to the decrease in oxygen concentration by a marked increase in the percentage of oxygen extracted (Fig. 11 and Table 3). This suggests an internal physiological change involving an increased efficiency of the blood in oxygen extraction.

(ii) *The effect of carbon dioxide and* pH

When carbon dioxide is bubbled through sea water, the water rapidly becomes acid, whilst the oxygen concentration is still not greatly diminished. In one typical experiment at 15° C. the oxygen concentration fell from 5·4 to 4·8 ml./l. whilst acidity increased from pH 8·3 to 6·0. The pulsation of the respiratory appendages and ventilation of the gills were found to cease at a pH of around 6·5. If excess carbon dioxide was blown off by aeration, respiratory movements recommenced at about the same pH. Alteration of the pH progressively from 8·5 to 7·0 evoked no permanent response in the rate of ventilation of the gills. However, the latter usually became erratic and diminished as the acidity increased beyond about pH 7·0.

Fox & Johnson (1934) found that the scaphognathite beat in the crayfish, *Astacus fluviatilis*, was unaffected by a rise in the carbon dioxide tension of the environment. Johnson (1936) reports that the scaphognathite beat in *Carcinus maenas* is very irregular and is apparently independent of the gas (oxygen and carbon dioxide) content of the medium. In the present experiments, within the range of acidity down to pH 7·0, the reactions of *Homarus vulgaris* to an increase of carbon dioxide were in agreement with the above results (Table 4).

Table 4. *The rate of scaphognathite beat, in a female lobster of weight* 360 g., *at* 15° C. *over the range* pH 8·3–6·7

pH	8·3	8·3	8·3	7·5	7·5	7·0	7·0	6·7
Scaphognathite (beats/min.)	104	101	99	102	100	71	106	101

The values of scaphognathite beat are each the mean of ten determinations. The standard error of each value is ±3·0 (D.F. = 72).

(iii) *The effect of temperature*

A series of experiments, by the method B, was carried out in which the temperature of the medium was varied whilst the oxygen concentration in sea water was maintained approximately constant.

Fig. 12 shows the relationship between oxygen uptake by the gills and temperature, in the case of a female lobster of weight 345 g., captured in January 1953, together with the corresponding rates of gill ventilation and percentages of oxygen extraction. The oxygen uptake over the temperature range of 5–21° C. is directly proportional to temperature. Both the rate of ventilation and the percentage extraction are also directly proportional to temperature. The fall in oxygen uptake with decreasing temperature is effected principally by a fall in the rate of ventilation of the gills, but a decrease in the percentage of oxygen extracted also contributes. Both these reactions may, in large measure if not entirely, be attributable to the direct effect of temperature on metabolism. Increased metabolic activity with increasing temperature would be expected to increase the frequency of the scaphognathite beat and to increase the rate of circulation of the blood.

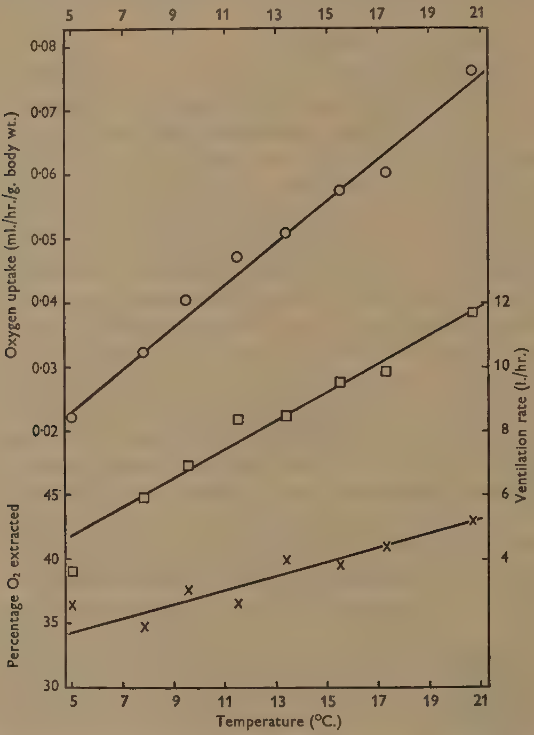

Fig. 12. The effect of temperature on the oxygen uptake by the gills, the rate of gill ventilation and the percentage of extraction of oxygen by a lobster, weight 345 g., in sea water of uniform oxygen concentration 5·3 ml./l. Throughout this experiment temperature was progressively reduced.

In order to determine to what extent a state of decreased oxygen availability varies the normal reaction to temperature, an experiment by the method B, similar to that described above, was carried out using rubber tubing of internal diameter 0·4 mm., thereby impeding the ventilation of the gills. The lobster used in this experiment was a female of weight 473 g., and was caught during June 1952. Fig. 13 shows the relationship between oxygen uptake and temperature, together with the corresponding data for rate of ventilation and percentage extraction. This experiment was begun at the top of the temperature scale, the temperature being reduced.

As in the experiment where the rate of ventilation was unrestricted, the oxygen uptake is directly proportional to temperature, although only at about half the unrestricted rate. Similarly, the rate of ventilation of the gills is directly proportional to temperature, although at an average rate of less than one-sixth 'normal'. However, the percentage extraction was 2·4 times the unrestricted level. In this experiment, furthermore, the degree of extraction rose from about 89 to 93 % throughout the duration of the experiment (6 hr. 9 min.), despite the fact that the temperature in the same interval was reduced from over 18° C. to less than 9° C. In this instance, notwithstanding the reduced oxygen uptake, the effect of temperature is much the same as in the previous experiment where oxygen uptake is unrestricted. The rate of ventilation of the gills, despite the relative lack of oxygen, varies with temperature in the same way as in the case of 'normal' oxygen uptake. This suggests that the scaphognathite is, in fact, reacting directly to temperature. Its rate of beat

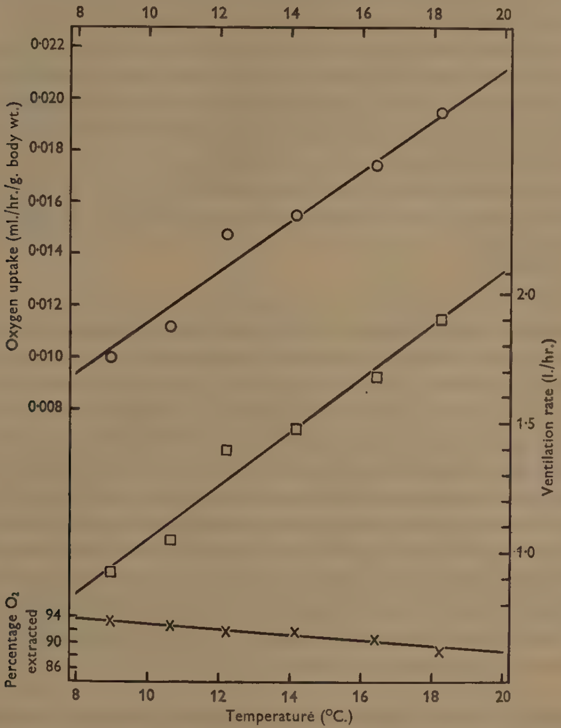

Fig. 13. The effect of temperature of the oxygen uptake by the gills, the rate of gill ventilation and the percentage of extraction of oxygen by a lobster, weight 473 g. In this experiment the rate of ventilation was artificially restricted. The sea water was maintained at a uniform oxygen concentration of 5·6 ml./l. and the temperature was progressively reduced. Compare with Fig. 12.

appears not to be influenced significantly by the reduced rate of oxygen uptake. This conclusion is supported by the results obtained in experiments (p. 242) in which the oxygen concentration of the medium was reduced. A decrease in the ventilation rate of the gills would be expected to result in an increase in the extraction rate for purely mechanical reasons. The high initial rate of extraction may therefore in part be attributable to the low ventilation rate. On the other hand, with decreasing temperature and consequent decrease in metabolism, the rate of circulation of the blood would be expected to fall, as did the rate of ventilation of the gills. The fact that the percentage extraction rose whilst the temperature was decreasing, in contrast to the experiment in which oxygen uptake was unrestricted, implies that there was a progressive building up within the animal of an oxygen deficiency and that this was reflected in a progressively increased efficiency in oxygen extraction of either the blood or the blood system, or both.

The oxygen uptake by the gills in this experiment is roughly comparable to that obtaining at an oxygen concentration of about 1 ml./l. as shown in run-down experiments (cf. Fig. 7).

(iv) *The maximum oxygen uptake*

It has been seen that a decrease in the oxygen concentration of the medium or a decrease in the rate of ventilation of the gills results in a decrease in oxygen uptake by the lobster. Conversely, experiments were carried out in which an increase of oxygen was made available in order, if possible, to establish an upper limit of the oxygen requirements. In one such experiment, by the method B, after determination of the normal oxygen uptake, the outlet end of the rubber tubing delivering the exhalant water was lowered and maintained at a level 7 cm. below the level of the tank water. Similarly, water samples for analysis were collected with this same degree of siphoning. In this way the rate of ventilation of the gills was artificially increased. The experiment was carried out at 15° C. and the oxygen concentration of the tank water maintained nearly constant at an average level of 5·73 ml./l. Data relating to a male lobster of weight 322 g., captured in March 1953 and typical for these experiments, are given in Table 5.

Table 5. *The effects of increased ventilation rate on oxygen uptake*

	Non-accelerated		Accelerated (7 cm.)		
Duration of accelerated ventilation (min.)	—	—	22	32	42
Rate of ventilation (l./hr.)	9·59	9·35	17·50	17·68	17·52
Percentage oxygen extraction	31·50	31·66	19·01	19·54	19·07
Oxygen uptake by the gills (ml./hr./g. body weight)	0·054	0·053	0·059	0·061	0·060

Whilst a decrease in the rate of ventilation of the gills has a very pronounced effect on the oxygen uptake, the above results indicate that a considerable increase in the rate of ventilation of the gills is not accompanied by any substantial increase in oxygen uptake. It can reasonably be assumed that the maximum oxygen requirement of the lobster is not significantly in excess of the normal oxygen uptake from well-aerated sea water, that is water in equilibrium with air. It should be noted the above results do not show how far acclimatization may be an important factor in establishing the 'normal' level of oxygen uptake.

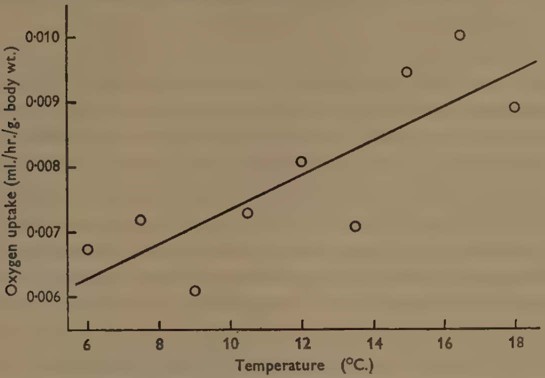

Fig. 14. The effect of temperature on the oxygen uptake by a lobster, weight 484 g., from air. ($P < 0{\cdot}01$.)

(*c*) *The oxygen uptake in air*

Fig. 14 shows the relationship between temperature and oxygen uptake by a lobster over sea water in carbon dioxide free air. These results, which are typical of the three series carried out, relate to a female of 484 g., caught during May 1952. Oxygen uptake is directly proportional to temperature over the experimental range 6–18° C. The oxygen uptake is, however, less than one-seventh of the corresponding uptake in well-aerated sea water. Since in these experiments the surface of the animal remains moist, diffusion of air to the gills will be very restricted. It seems possible, therefore, that the oxygen uptake in these experiments will, in part, be effected through the abdominal swimmerets, which, as seen in § 3(*a*)(iv), have a respiratory function.

The determination at each experimental temperature occupied about 5 hr., and in view of the low level of oxygen uptake, it seems reasonable to suppose that the value obtained represents the level of uptake with the physiology of the animal adjusted to the appropriate high degree of oxygen want. Under such circumstances the decrease in oxygen uptake with decrease of temperature, as in the experiment, by the method B, in which the rate of ventilation was restricted (Fig. 13), is due to the decrease in the rate of metabolism being reflected in the rate of transport of oxygen within the animal.

IV. DISCUSSION

It has been possible in these experiments to study only a limited and highly selected range of material. Lobsters tend to be sluggish at temperatures of 5° C. and below. It seems likely that winter-caught animals will represent only the more active individuals from the population. Some degree of selection will operate in capture at any season. For instance, newly moulted lobsters are very seldom taken in traps. The investigations are further restricted to a narrow size range and by the elimination of lobsters carrying spawn and specimens showing signs of recent moulting.

In certain crustaceans, Fox (1936) and Edwards & Irving (1943) have demonstrated the adjustment of the oxygen consumption to a prevailing temperature. This results in a seasonal variation. The procedures adopted in the present experiments are not adapted for the investigation of seasonal fluctuations in oxygen uptake.

Although investigations were conducted throughout the year, a seasonal fluctuation was not apparent. This may be due to the small numbers used. In addition, the overnight acclimatization to the initial experimental temperature will tend to mask any such effect. McLeese working with Wilder (personal communication) finds that the American lobster (*Homarus americanus*) undergoes a slow physiological adaptation to the immediate environmental conditions. As suggested by Bosworth *et al.* (1936) the readjustment to change in environmental conditions may, in terms of oxygen uptake, even be effective, to some extent, over the duration of an experiment. It certainly will be a factor where the experiments are repeated on succeeding days at different temperatures. It is possible that it accounts, in part, for the difference, noticeable at lower temperatures, between the variation in oxygen uptake with temperature as determined by the method A (Fig. 7) and by the method B (Fig. 12).

The extensive period of experiments in investigating the effect of temperature by the method A and in air is a considerable limitation since, as already pointed out, the lobster is readjusted to a different temperature every day. Whilst the effect of this on oxygen uptake may not be proportionately large, its extent is an unknown. Similarly, the effects upon the animal of previous experiments cannot be assessed. The method B largely avoids these limitations. The latter technique, however, only measures gill uptake. On the other hand, this represents the major part of the total. Furthermore, a figure for total uptake could reasonably be taken as the gill uptake augmented by 3 %. The method B has a further advantage in that it affords data on the rate of ventilation and on utilization. The broad agreement between results from both the methods A and B indicates that the limitations of either are not serious.

In *Astacus* the rate of scaphognathite beat increases with rise in temperature. The present results on ventilation rates of the gills of *Homarus vulgaris* show a similar relationship. Fox & Johnson (1934) and Johnson (1936), reporting on the rate of respiratory movement in various crustacea, have demonstrated the variability of response between species in relation to decreased oxygen tension and to increased concentration of carbon dioxide in the medium. In the present experiments, where acount was made of the rate of respiratory movements, no reaction was found either to decreased oxygen or to increased carbon dioxide except for an inhibitory effect of the latter due to the acidity when this increased beyond about pH 7·0. These results are in agreement with observations on the rate of gill ventilation in experiments by the method B. Prosser (1950), on the other hand, quotes a positive response in scaphognathite movement to both oxygen want and to increase of carbon dioxide. The experimental details, however, are not available.

Amberson *et al.* (1924) state: 'Identical curves [for experiments by the method A] are obtained when the same animals are placed in sea water with different carbon dioxide tensions. The production of carbon dioxide does cause a slight change in the pH of the water, but we find that changes in carbon dioxide tensions much exceeding this amount have no effect upon the rate of oxygen consumption.' Since increased carbon dioxide does not affect oxygen uptake, it seems unlikely that it will result in an increased rate of ventilation. The percentage oxygen extraction in sea water of oxygen concentration 5·33 ml./l. at 15° C. is about 40% (Fig. 12). The degree of utilization in *H. vulgaris* is therefore not high. Considered from the physical aspect, and apart from any change in the efficiency of the blood system, a considerable increase in ventilation rate will result in a relatively small increase in oxygen uptake. The percentage extraction rate will fall as the ventilation rate increases tending partially to offset the effect of the latter on oxygen uptake. The weight of evidence supports the conclusion that in *H. vulgaris* the ventilation rate, at any rate over a period of some hours, is not significantly affected by either oxygen want or carbon dioxide increase.

A rise in temperature will result in an increase in the metabolic rate. In normal circumstances this will be reflected in a corresponding increase in the respiratory movements, leading to an increase in the rate of ventilation. The increase in blood circulation rate and the rate of oxygen diffusion, also consequent upon the increase in the metabolic rate, will increase utilization. In consequence, the percentage extraction rate should be maintained. However, as the resistance to the flow of water over the gills is considerable, and increases relatively as the rate of flow increases, a given increase in the driving force of the respiratory movements will not lead to a proportionate increase in the ventilation rate. On the other hand, with an increase in temperature the increased circulation rate and more rapid oxygen diffusion will roughly correspond with the increase in the metabolic activity. Hence, the percentage of oxygen extracted by the gills will tend to rise. This explanation is probably an over-simplification but it does adequately account for the experimentally observed results (Fig. 12).

The rise in metabolic activity accompanying a rise in temperature should be similar to the above even when the ventilation rate of the gills is restricted. The converse should apply where there is a fall in temperature, as in the experiment illustrated in Fig. 13. Utilization would be expected to fall with decreasing temperature. The percentage oxygen extraction, however, tends to rise progressively as the experiment proceeds, notwithstanding the fall in temperature and the high initial value of over 88%. This is presumably attributable to a progressive increase in the efficiency of oxygen transportation within the animal in response to the developing oxygen want. The conclusion is supported by the results of experiments by the method B in which the oxygen concentration of the medium was depleted by means of nitrogen (Fig. 11, Table 3). In these experiments, in which the temperature was constant, the ventilation rate was unrestricted and remained at a roughly constant level. As the oxygen concentration of the medium was reduced, the percentage of oxygen extracted rose considerably from the initial value of about 30%. It appears that this response to oxygen want represents a change in the respiratory efficiency of the blood itself.

Redfield & Ingalls (1933) have shown that in a blood serum from *H. americanus* the fraction of haemocyanin present as oxyhaemocyanin increases very considerably over the range pH 6·4–8·8. Cole (1940) quotes 7·45 and 7·61 as representative values for the pH of lobster blood. It could well be that the observed response to internal oxygen deficiency operates through a change in the pH of the blood. Certainly, it would appear that in *H. vulgaris* some change in the efficiency of the blood circulation is the main compensatory response to changes in the availability of oxygen.

Notwithstanding these possibilities, in a medium at constant temperature which is being depleted of oxygen, the lobster is unable to maintain its oxygen uptake. Under these circumstances it must presumably build up an oxygen debt or supplement the aerobic processes. This problem is outside the scope of the present experiments, although the results do suggest that the adaptability of the lobster in this respect is considerable.

V. SUMMARY

1. In sea water the oxygen uptake of *Homarus vulgaris* is directly proportional to the oxygen concentration. The relationship applies over the temperature range 6–18° C.

2. Within specified limits of size and condition, oxygen uptake is the same for both sexes.

3. The relative oxygen uptake in sea water decreases as the weight of the animal increases.

4. Oxygen uptake in sea water is effected mainly through the gills. The abdominal swimmerets, however, also serve in respiration and account for approximately 3% of the total oxygen uptake.

5. In sea water of constant oxygen tension, oxygen uptake increases with increasing temperature.

6. Increase in oxygen uptake with temperature in sea water is mainly brought about by an increase in the gill ventilation rate. In addition, the degree of utilization increases. The relationship is a direct reflexion of the increased metabolic activity.

7. The ventilation rate of gills is unaffected by a decrease of oxygen.

8. The percentage of oxygen extracted by the gills increases as the oxygen concentration of the medium decreases.

9. Under the influence of carbon dioxide respiratory movements become retarded at acidities greater than about pH 7·0 and are completely inhibited at around pH 6·5. At acidities less than pH 7·0 changes in the carbon dioxide concentration are without effect on the rate of the respiratory movements.

10. The oxygen uptake in air, notwithstanding its low level, is directly proportional to temperature.

11. The significance of the above results in relation to the respiratory functions of the blood is discussed.

Sincere thanks are due to Prof. J. Gray, F.R.S., for suggesting this research and for his continuing interest, and to Prof. H. Munro Fox, F.R.S., also to Mr J. A. Pope, of this laboratory, for his considerable assistance in the statistical treatment of the results.

REFERENCES

ALLEE, W. C. & OESTING, R. (1934). *Physiol. Zoöl.* **7**, 509.
AMBERSON, W. R., MAYERSON, H. S. & SCOTT, W. J. (1924). *J. Gen. Physiol.* **7**, 171.
ANON. (1952). *Practical Hints for Lobster Fishermen.* Edinburgh: H.M.S.O.
BOSWORTH, M. W., O'BRIEN, H. & AMBERSON, W. R. (1936). *J. Cell. Comp. Physiol.*, **9**, 77.
BRUNOW, H. (1911). *Z. allg. Physiol.* **12**, 215.
COLE, W. H. (1940). *J. Gen. Physiol.* **23**, 575.
DIXON, M. (1943). *Manometric Methods*, 2nd ed. Cambridge University Press.
EDWARDS, G. A. & IRVING, L. (1943). *J. Cell. Comp. Physiol.*, **21**, 169.
FOX, H. MUNRO (1936). *Proc. Zool. Soc. Lond.* **106**, 945.
FOX, H. MUNRO & JOHNSON, M. L. (1934). *J. Exp. Biol.* **11**, 1.
JACOBSEN, J. P. (1921). *Bull. Inst. océanogr. Monaco*, No. 390.
JOHNSON, M. L. (1936). *J. Exp. Biol.* **13**, 467.
KEYS, A. B. (1930). *Biol. Bull., Woods Hole*, **59**, 187.
KROGH, A. (1916). *The Respiratory Exchange of Animals and Man.* London: Longmans, Green and Co.
ORR, A. P. (1926). *J. Mar. Biol. Ass. U.K.* **14**, 55.
PROSSER, C. L. ED. (1950). *Comparative Animal Physiology.* Philadelphia and London: Saunders.
REDFIELD, A. C. & INGALLS, E. N. (1933). *J. Cell. Comp. Physiol.* **3**, 169.
ROGERS, C. G. (1938). *Textbook of Comparative Physiology*, 2nd ed. New York and London: McGraw-Hill.
WEYMOUTH, F. W., CRISMON, J. M., HALL, V. E., BELDING, H. S. & FIELD, J. (1944). *Physiol. Zoöl.* **17**, 50.
WINKLER, L. W. (1888). *Ber. dtsch. chem. Ges.* **21**, 2843.
ZEUTHEN, E. (1953). *Quart. Rev. Biol.* **28**, 1.

THE PHYSIOLOGY OF SEA-URCHIN SPERMATOZOA

ACTION OF VERSENE

BY LORD ROTHSCHILD AND A. TYLER*

Department of Zoology, University of Cambridge,
and the Marine Station, Millport

(*Received* 20 *July* 1953)

INTRODUCTION

A description of the physiology of sea-urchin spermatozoa requires, among other things, an explanation of two special characteristics which these spermatozoa possess. First, immediately after dilution, the O_2 uptake per spermatozoon is higher in a dilute than in a dense suspension. Secondly, the decline in sperm motility and fertilizing capacity is more rapid in a dilute than in a dense suspension. The first of these characteristics can be reversed by diethyldithiocarbamate (Rothschild, 1950) and altered by copper ions (Rothschild & Tuft, 1950), while the second can be profoundly influenced by amino-acids (Tyler & Atkinson, 1950) and metal-chelating agents, including diethyldithiocarbamate (Tyler, 1953). Up till now there has been a tendency for these phenomena to be studied separately and on different species of sea-urchin spermatozoa. It was therefore decided to examine the Dilution Effect as a whole, using the spermatozoa of *Echinus esculentus*.

We had both independently come to the conclusion that the Dilution Effect might be explained by the presence of trace elements, in particular copper, in the sea water. We therefore decided to examine the effect of the well-known chelating agent versene on the metabolism, motility and fertilizing capacity of these spermatozoa. This reagent was selected because there is some evidence that it is biologically innocuous (Hutner, Provasoli, Schatz & Haskins, 1950), while diethyldithiocarbamate, apart from being oxidized by cytochrome to tetraethyldithiocarbamyl disulphide, which itself may be an inhibitor, could exert a narcotic action, though there is evidence against this possibility (Rothschild, 1950).

The study of mammalian spermatozoa is sometimes facilitated by 'washing' the semen before the experiment, which involves repeated centrifugation and replacement of the seminal plasma by solutions of known composition. Up till now this procedure has proved to be difficult to apply to sea-urchin semen which, by virtue of its high sperm density, is far more viscous than bull or ram semen. Because, however, of the possibility that sea-urchin seminal plasma might exert a chelating effect on trace elements in the sea water, we have re-examined the feasibility of centrifugation and washing, and the possibility that dilution of the seminal plasma may be responsible for the Dilution Effect.

---

* Professor of Embryology, California Institute of Technology, and Fellow of the John Simon Guggenheim Memorial Foundation.

MATERIAL AND METHODS

Semen and spermatozoa of *Echinus esculentus*. Outside filtered sea water was used in all experiments, in particular because Cleland (1953) showed that Millport tap sea water may be contaminated with heavy metals and that they interfere with the fertilization reaction in sea-urchin eggs. O_2 uptake was measured by the usual Barcroft-Warburg technique, at 15° C and with air in the gas phase.

RESULTS

Unwashed spermatozoa. The effect of versene on the initial phases of sperm metabolism is shown in Fig. 1. The O_2 uptake was measured for 40 min., after which sea water containing versene at concentrations ranging from 10^{-6} to 10^{-3} M (pH of sea water adjusted to 8·1) was added. The following points may be noted in Fig. 1. First, when sea water without versene was added, there was the usual Dilution Effect, but the effect was rather small. Although large respiratory Dilution Effects were observed on a few occasions during the season, in general the increases in O_2 uptake on dilution were smaller than in previous years. One possible explanation of this difference is that Millport sea water contained 0·0012 μg./Cu/ml. this year,* while on the last occasion the measurements were made (Barnes & Rothschild, 1950), it contained 0·0064 μg. Cu/ml. In the presence of 10^{-6} and 10^{-5} M versene in sea water there was still a small Dilution Effect, but in 10^{-4} and 10^{-3} M versene in sea water, the Dilution Effect was almost completely abolished and, particularly in the stronger solution, there was a marked inhibition of O_2 uptake. After 7 hr. the suspension without versene had fallen below those containing versene, while after 17 hr., apart from some increase probably due to the multiplication of bacteria, the rates of O_2 uptake were still about the same as after 7 hr. There was, however, a profound difference in the conditions of the suspensions after 17 hr., those containing 10^{-4} and 10^{-3} M versene having a significant proportion of active spermatozoa in them, while those containing 10^{-6} or 10^{-5} M versene, or no versene, contained virtually no active spermatozoa. This 'protective' effect of versene was not restricted to motility, though it is well known that spermatozoa can and do lose fertilizing capacity without any corresponding loss of motility. The fertilizing capacities of the suspensions were determined by making serial dilutions of the suspensions from each manometer vessel after 17 hr. incubation, and adding the same number of eggs to each, immediately after making the dilution. The percentage of fertilized eggs was noted and the results are given in Table 1, from which it will be seen that the suspensions containing the higher concentrations of versene maintained their fertilizing capacity to about the same extent as their motility, while when no versene was present, fertilizing capacity was virtually zero at all the dilutions tried. Reference to Table 1 shows, for example, that a suspension, density $9{\cdot}3 \times 10^4$/ml., in 10^{-3} M versene in sea water has more than 125 times the fertilizing capacity of a suspension with a higher sperm density, $1{\cdot}2 \times 10^7$/ml., without versene. Similar results were obtained in four other experiments of the same type.

---

* We are indebted to Dr H. Barnes for estimation of the copper in sea water.

Apart from the experiments involving the simultaneous examination of metabolism and viability, we did five other experiments, in which only motility and fertilizing capacity were estimated. These confirmed the extraordinary effect of versene in delaying the senescence of these spermatozoa. On the average, a 1 % suspension of semen in sea water (i.e. app. 2 × 10^8/ml.) contained no living spermatozoa after about 20 hr. incubation at 15° C. Spermatozoa in a 0·1 % suspension were all dead in about 8 hr., and in a 0·01 % suspension in about 2 hr. The comparable figures when the sea water contained 10^{-3} M versene were 72 ($3\frac{1}{2}$ times as long), 48 (×6)

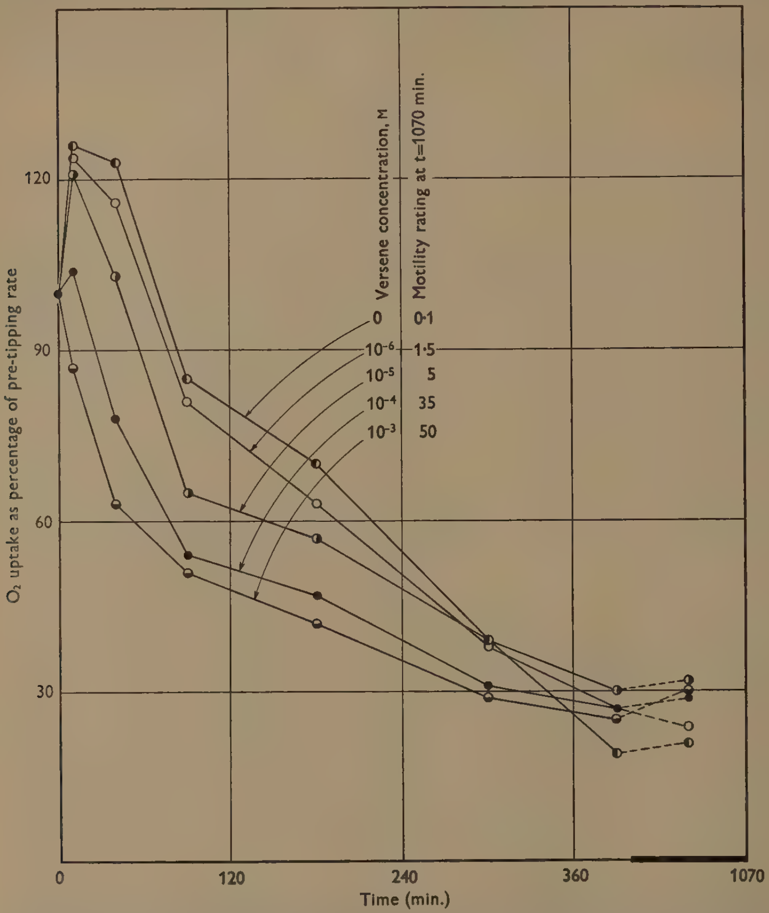

Fig. 1. Effect of versene on O_2 uptake of sea-urchin spermatozoa. Average O_2 uptake, in μl. O_2/hr., in all suspensions, immediately before addition of reagents, 55·4, S.E. 3·4. Sperm density before addition of reagents, 5·80 × 10^8/ml.; after addition of reagents, 2·90 × 10^8/ml.

and 24 (× 12) hr. In several of these experiments the motility and fertilizing capacity of spermatozoa in 1 and 0·1 % suspensions in versene-sea water were about the same after 48 hr. incubation as at the beginning of the experiment, while those in ordinary sea water had all died in less than 12 hr.

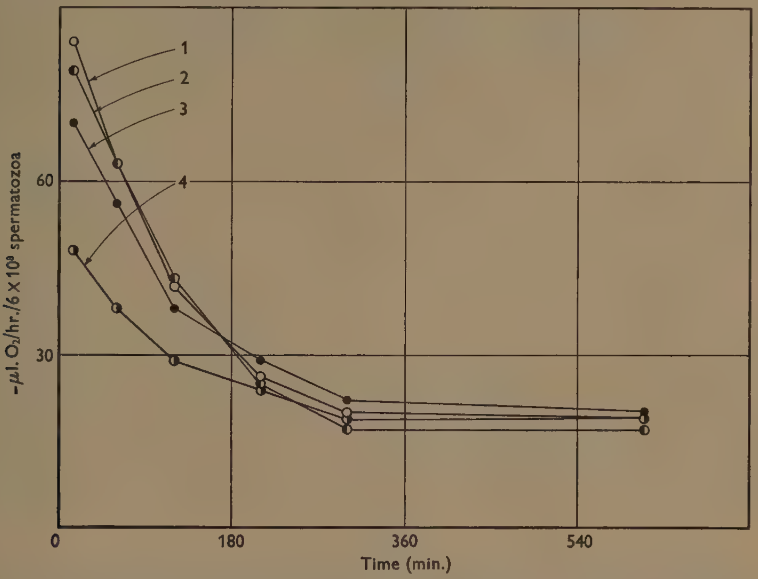

Fig. 2. Effect of washing on O_2 uptake of sea-urchin spermatozoa. (1) Unwashed; (2) washed and re-suspended in supernatant; (3) washed and re-suspended in sea water; (4) washed and re-suspended in sea water containing 10^{-3} M versene. For further details, see text.

Washed spermatozoa. In these experiments a comparison was made between (1) semen diluted in sea water (1/20); (2) semen diluted in sea water (1/20) and centrifuged twice for 3 min. at 12,000 g. with re-suspension in its own supernatant; (3) semen diluted with sea water (1/20) and centrifuged twice for 3 min. at 12,000 g. with replacement of its supernatant by sea water; and (4) semen diluted with sea water (1/20) and centrifuged twice for 3 min. at 12,000 g. with replacement of its supernatant by sea water containing 10^{-3} M versene (pH adjusted to 8·1). Fig. 2 shows that this can be achieved without significant damage to the spermatozoa and that the removal of seminal plasma does not materially alter the picture of the effect of versene in Fig. 1. The inhibitory action of versene on the initial O_2 uptake is, however, very marked, even though this 'inhibitor' greatly improved the fertilizing capacity of the suspension. Table 2 also shows that removal of seminal plasma sharply reduces the fertilizing capacity of the suspension, but the addition of versene prevents this.

Effect of seminal plasma. Respiration, duration of motility and fertilizing capacity were examined in suspensions containing different numbers of spermatozoa but the same concentration of seminal plasma as the densest suspension. The respirometric findings were the same as those of Hayashi (1945, 1946) and Rothschild (1948, 1950). Briefly, dilution of seminal plasma is not responsible for the respiratory symptoms of the Dilution Effect. The effect of seminal plasma on fertilizing capacity is shown in Table 3. The motility estimates gave similar but, for obvious reasons, less quantitative results. Table 3 shows that, as in the case of the respiratory Dilution Effect, the reduced ratio of seminal plasma to sea water in ordinary dilute suspensions cannot explain their increased rate of senescence.

Table 1. *Fertilizing capacity, expressed as percentage eggs fertilized in test fertilization of sea-urchin spermatozoa* (Echinus esculentus) *after incubation for* 1020 *min. in versene-containing sea water at* 15° C. 0·05 *ml. unfertilized egg suspension* (*approx.* 200) *were added to* 4 *ml. of sperm suspension taken from the flasks used in the experiment in Fig.* 1 *and diluted as shown*

No. of sperm/ml.	Molar concentration of versene in sea water					
	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	0 (fresh unincubated semen)
$1{\cdot}2 \times 10^{7}$	5	75	90	95	100	100
$2{\cdot}3 \times 10^{6}$	1	25	75	50	100	100
$4{\cdot}6 \times 10^{5}$	0	10	50	75	90	100
$9{\cdot}3 \times 10^{4}$	0	0	2	25	50	95

Table 2. *Fertilizing capacity, expressed as percentage eggs fertilized in test fertilization, of sea-urchin spermatozoa* (Echinus esculentus) *after incubation for* 1000 *min at* 15° C. 0·1 *ml. unfertilized egg suspension* (*approx.* 300) *were added to* 4 *ml. of sperm suspensions taken from the flasks used in the experiment in Fig.* 2 *and diluted as shown:* (1) *uncentrifuged;* (2) *centrifuged and re-suspended;* (3) *centrifuged and re-suspended in sea water;* (4) *centrifuged and re-suspended in sea water containing* 10^{-3} M *versene*

No. of sperm/ml.	(1)	(2)	(3)	(4)
$7{\cdot}5 \times 10^{6} - 3{\cdot}0 \times 10^{5}$	100	100	100	100
$6{\cdot}0 \times 10^{4}$	100	100	50	100
$1{\cdot}2 \times 10^{4}$	90	85	15	90

Table 3. *Fertilizing capacity, expressed as percentage eggs fertilized in test fertilization of sea-urchin spermatozoa* (Echinus esculentus) *after* 6 *hr. incubation at* 15° C.: (1) *sperm density during incubation,* $8{\cdot}38 \times 10^{8}$ *ml.; seminal plasma concentration,* 2·6 %. (2) $4{\cdot}19 \times 10^{8}$ *sperm/ml.; plasma,* 2·6 %. (3) $2{\cdot}09 \times 10^{8}$ *sperm/ml.; plasma,* 2·6 %. (4) $1{\cdot}05 \times 10^{8}$ *sperm/ml.; plasma,* 2·6 %

No. of sperm/ml.	(1)	(2)	(3)	(4)
$8{\cdot}4 \times 10^{4}$	100	100	100	100
$1{\cdot}7 \times 10^{4}$	100	100	100	100
$3{\cdot}4 \times 10^{3}$	100	95	50	10
$6{\cdot}8 \times 10^{2}$	100	70	5	0

DISCUSSION

The experiments described in this paper support the hypothesis that the Dilution Effect is at any rate in part caused by heavy metals, which for the moment will be called copper, normally present in sea water. Suppose, for example, a suspension of sea-urchin spermatozoa, sperm density 6×10^8/ml., is diluted with sea water to a density of 3×10^8/ml. According to the hypothesis, addition of sea water causes an increase in the concentration of copper per spermatozoon in the suspension. The copper is toxic and its effect is evidenced after its addition via the diluting sea water in two ways: first, by an immediate increase, followed by a fall, in the O_2 uptake of the suspension; and secondly, by a higher senescence rate, as shown by an increase in the number of inactive or dead spermatozoa in the suspension and by a decrease in its fertilizing capacity. It might be thought that a simple way of testing this hypothesis would be to add sufficient amounts of an innocuous chelating agent, like versene, to the sea water, to bind all the copper present. When, however, such an experiment is done, it is found that concentrations of versene ranging from 10^{-6} to 10^{-3} M exert a graded inhibition or reversal of the toxic effect of sea water. 10^{-6} M versene should be sufficient to bind, not only the copper, but also other trace metals in sea water. An increased concentration of versene might therefore not be expected to exert any additional 'protective' effect on the spermatozoa. Possible interpretations of this graded inhibition, which also occurs when glycine is substituted for versene (Tyler & Rothschild, 1951), are that versene does not combine in a 'simple' way with the copper in sea water; or that there exists some form of dynamic equilibrium between the copper, the spermatozoa, and the versene, which accounts for the apparently non-stoichiometric relationship between the concentrations of copper and versene in the sea water. Sea-urchin spermatozoa are known to combine strongly with copper, there being about 300 times as much of this element in the spermatozoa of *E. esculentus* as in sea water (Barnes & Rothschild, 1950). Seminal plasma, on the other hand, contains much less copper than the spermatozoa; one would not, therefore, expect it to be so efficient a protective agent as versene and this has been confirmed in the experiments described above. At the same time it is surprising that versene concentrations as high as 10^{-3} M are needed to achieve what, even at that concentration, may not be a maximum protective effect.

So far it has been assumed that copper is the metal in sea water responsible for these effects. The evidence that zinc may be involved is very slight, being limited to a sentence in Rothschild & Tuft's paper (1950) that $ZnCl_2$ stimulates the O_2 uptake of sea-urchin spermatozoa. In the same paragraph it is said that iron has no stimulating effect. Calcium, which is chelated by versene, is known not to be the causative agent, as its concentration can be varied over wide limits without the spermatozoa being affected (Tyler, 1953). Bearing in mind that the addition of copper to dense sperm suspensions can increase their O_2 uptake to the level of dilute suspensions, while the reverse process can be achieved with diethyldithiocarbamate (Rothschild, 1950), it seems reasonable to assume for the time being that the heavy metal in sea water responsible for these effects is copper.

In this paper we have adopted the view that the copper in sea water is toxic to spermatozoa, which implies that in this respect sea-urchin spermatozoa are poorly adapted to their post-spawning environment, if it is assumed that copper has always been present in sea water. For except in the rare event of almost complete absence of water movements, the dilution of semen after spawning will be sufficient to 'injure' the spermatozoa through the operation of the Dilution Effect. One of us (Rothschild, 1950) interpreted earlier respiration experiments in a different way—on the basis that each spermatozoon requires a certain amount of copper in the external medium. This concept has certain merits in suggesting a useful role for copper in sea water, in contradistinction to the implications of the experiments described in this paper. Further work is needed to find out if the correct interpretation of the Dilution Effect lies somewhere between these two points of view.

SUMMARY

1. The effect of adding sea water containing different concentrations of versene to suspensions of sea-urchin spermatozoa (*Echinus esculentus*) has been investigated, as regards their respiration, motility and fertilizing capacity.

2. The respiratory Dilution Effect is progressively reduced and finally abolished when, instead of sea water, sea water containing 10^{-6}, 10^{-5}, 10^{-4} or 10^{-3} M versene is added to sperm suspensions.

3. At the same time versene greatly delays the senescence of the spermatozoa, both as regards their motility and fertilizing capacity. For example, after seventeen hours, a 10^5 sperm/ml. suspension in sea water containing 10^{-3} M versene has 125 times the fertilizing capacity of a suspension containing 10^7 sperm/ml. without versene.

4. The change in the ratio of seminal plasma to sea water which occurs when a dense suspension is diluted does not explain the Dilution Effect.

5. These results are discussed in relation to the hypothesis which accounts for the Dilution Effect in terms of trace metals, particularly copper, normally occurring in sea water, and the amounts available per spermatozoon under various conditions of dilution.

One of us (T.) is greatly indebted to Prof. Sir James Gray, F.R.S., for his hospitality and kindness in the Department of Zoology, University of Cambridge; the other (R.) is indebted to the Medical Research Council for the provision of an assistant.

REFERENCES

BARNES, H. & ROTHSCHILD, LORD (1950). A note on the copper content of sea-urchin semen and sea water. *J. Exp. Biol.* **27**, 123–5.

CLELAND, K. W. (1953). Heavy metals, fertilization and cleavage in the eggs of *Psammechinus miliaris*. *Exp. Cell Res.* **4**, 246–8.

HAYASHI, T. (1945). Dilution medium and survival of the spermatozoa of *Arbacia punctulata*. I. Effect of the medium on fertilizing power. *Biol. Bull., Woods Hole*, **89**, 162–79.

HAYASHI, T. (1946). Dilution medium and survival of the spermatozoa of *Arbacia punctulata*. II. Effect of the medium on respiration. *Biol. Bull., Woods Hole*, **90**, 177–87.

Hutner, S. H., Provasoli, L., Schatz, A. & Haskins, C. P. (1950). Some approaches to the study of the metals in the metabolism of micro-organisms. *Proc. Amer. Phil. Soc.* **94**, 152–70.

Rothschild, Lord (1948). The physiology of sea-urchin spermatozoa. Senescence and the Dilution Effect. *J. Exp. Biol.* **25**, 353–68.

Rothschild, Lord (1950). The respiration of sea-urchin spermatozoa. *J. Exp. Biol.* **27**, 420–36.

Rothschild, Lord & Tuft, P. H. (1950). The physiology of sea-urchin spermatozoa. The Dilution Effect in relation to coppr and zinc. *J. Exp. Biol.* **27**, 59–72.

Tyler, A. (1953). Prolongation of life-span of sea-urchin spermatozoa, and improvement of the fertilization-reaction, by treatment of spermatozoa and eggs with metal-chelating agents (amino acids, versene, DEDTC, oxine, cupron). *Biol. Bull., Woods Hole*, **104**, 224–39.

Tyler, A. & Atkinson, E. (1950). Prolongation of the fertilizing capacity of sea-urchin spermatozoa by amino acids. *Science*, **112**, 783.

Tyler, A. & Rothschild, Lord (1951). Metabolism of sea-urchin spermatozoa and induced anaerobic motility in solutions of amino acids. *Proc. Soc. Exp. Biol., N.Y.*, **76**, 52–8.

CHANGES IN THE BLOOD POTASSIUM CONCENTRATION OF THE AFRICAN MIGRATORY LOCUST (*LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.) DURING FOOD DEPRIVATION, AND THE EFFECT ON NEUROMUSCULAR ACTIVITY

By G. HOYLE

Departments of Zoology, University College, London,
*and the University of Glasgow**

(*Received* 17 *September* 1953)

INTRODUCTION

The nerve and muscle fibre membranes of adult locusts, like those of other animals (Hodgkin, 1951), are in a state of electrical polarization which is reduced by increasing the concentration of potassium ions in the surrounding medium (Hoyle, 1953*a*). This change is reversible if there is no prolonged contact with the higher concentrations. The locust nerve trunks are surrounded by a sheath which is highly impermeable to potassium ions, and which may protect the axons from a high external potassium concentration which would otherwise impair or block conduction. The muscle fibres are not similarly protected; potassium ions in moderately high external concentration readily penetrate the thin tracheolated membranes which bind the muscle fibres together and depolarize them. The end-plate potential and muscle spike action potential (see del Castillo, Hoyle & Machne, 1953) are both reduced by raising the external potassium level and smaller contractions result when the muscles are then excited via the nerves (Fig. 1). It is not within the scope of the present paper to describe the physiological action of potassium ions in detail, and this will be done elsewhere, but it will be of value to point out that in a given muscle, exploration of a large number of the component fibres with the aid of intracellular capillary microelectrodes shows that allowing for a slower rate of diffusion to the innermost fibres, every fibre is affected in the same way, and that the reduced tension produced in higher concentrations of potassium represents the sum total of weaker contractions in each fibre and not a reduction in the number of active fibres. That the muscle fibres can contract at all when depolarized to a considerable extent as they are in say 50 mM. K/l., seems due to the combined effect of small contractions produced at a large number of nerve endings supplying each fibre.

The muscles are operated by 'slow' and 'fast' nerve fibres (Hoyle, 1953*b*, *c*), and potassium affects both these systems. The slow system is affected more than the fast, so that whereas the fast system twitch is reduced to about 50% maximum by 40 mM. K saline, the slow tension is reduced to about 30% maximum in the same medium. In all cases resting membrane potential, action potential, twitch tension, tetanus and slow fibre tension are all greatest when the bathing fluid contains zero potassium.

---

* Present address.

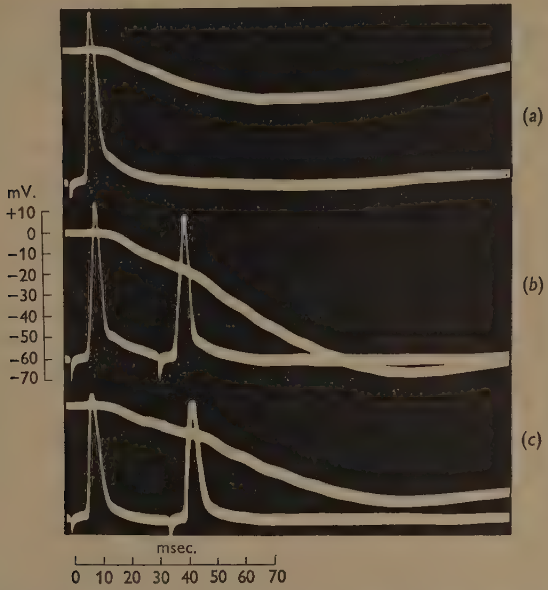

Fig. 1. Electrical and mechanical records of activity in the extensor tibialis muscle of an adult locust following single or paired stimulation of the 'fast' nerve trunk: (*a*) in 0 mM. K, (*b*) in 10 mM. K, (*c*) in 20 mM. K. In each record the upper trace is the mechanical response, increase of tension indicated downwards, while the lower trace is the trans-membrane electrical response of a single fibre, increasing positivity indicated upwards. Before records were taken the tip of the electrode was immersed in earthed saline and by adjustment of the vertical (Y) shift the two traces were brought into coincidence. The base-line of the upper trace thus indicates both zero tension and zero potential. The electrode was then inserted into the muscle fibre and when the record begins the lower trace indicates first the resting potential and subsequently the transients associated with activity.

The haemolymph of many herbivorous insects is known to contain concentrations of potassium of relatively great magnitude (table in Roeder, ed., 1953). If this potassium is in the freely ionized condition the muscles of the locust, when bathed in such a medium, could give only weak mechanical responses. This raises a number of problems, e.g.:

(1) What is the order of magnitude of potassium concentration in locust blood?

(2) Would this concentration of potassium (if high as in other insects) affect the muscle properties when compared with lower concentrations (i.e. is most of the potassium in the freely ionized condition)?

(3) Is the potassium concentration related to diet, for plant leaves have a high potassium content?

(4) Does starvation result in decreased blood potassium, for then the source of diet potassium is removed? If so, can natural changes in blood-potassium concentration be shown to affect the properties of the muscles?

These problems were not tackled in logical sequence, owing to the initial absence of facilities for measuring blood potassium. Problem (4) was tackled first, measurements being made of twitch tension of the extensor tibialis muscle (bathed in blood) of 5th-instar nymphs starved for various periods following grass feeding. As these tests showed that there was a substantial increase in the average twitch tension of this muscle during the first 7 hr after removal of food, the experiments described below (and also Ellis & Hoyle, 1954) were executed to supply answers to the other problems outlined above.

SODIUM AND POTASSIUM CONCENTRATIONS DURING FEEDING AND STARVATION

For these investigations only animals known to be in mid-5th instar or at least 7 days after the final moult, and giving evidence of being in good condition, were used. Recent work (in progress) has shown that great changes in the sodium and potassium contents of the haemolymph occur near the time of moulting. Sodium and potassium concentrations of whole haemolymph were determined with the aid of an Eel-flame photometer. Samples of equal volume (about 1 mm.^{3}) were drawn into the graduated portion of a haemocytometer pipette and then mixed with 2 ml. distilled water. The samples were obtained from a cut made dorsally in the thin cuticle between the pronotum and the thorax. Blood collected slowly in adults, rapidly in hoppers, and when a sufficient droplet had collected it was sucked into the pipette. Frequently considerable difficulty was experienced in extracting even 1 mm.^{3} from adults, especially when starved, and a few appeared to contain no blood at all! However, by giving the animals which were housed in a constant temperature room at 25° C. a supply of drinking water, the number of such bloodless animals was reduced. The diluted blood was sucked into the atomizer of the photometer and readings taken of sodium and potassium using standard filters. Test readings were made at intervals from standard samples of sodium and potassium chlorides for calibration.

Animals were separated before analysis into groups of ten similar animals each, one group was kept under the standard conditions in a humid atmosphere with an abundant supply of fresh grass to eat and water to drink. The other group was kept under similar conditions but in the absence of food, overnight. Blood was withdrawn and analysed the following morning; results of the analyses are shown in Table 1.

The results (Table 1) show that for hoppers of both the solitary-reared and gregarious-reared types the sodium concentration has about the same value in feeding forms. There is in both a reduction of 11–18 mM. Na during the foodless period. The potassium concentration of the two types is likewise similar, and both show a considerable loss (50%—16 mM.) during the foodless period. The animals were fed on common British grasses and especially on 'twitchgrass'—*Agropyron* *repens*. The area of this grass eaten by 5th-instar nymphs has been determined by Dr Ellis (unpublished observations). They eat an average of 25 cm.^{2} leaf area per animal per 12 hr. period under laboratory conditions at 30° C. The average potassium content of this grass, determined in terms of leaf area is 0·09 mg./cm.^{2} ± 2 % (see acknowledgements). A normal daily intake for a 5th-instar nymph is therefore of the order of 4·5 mg. K. Since the total blood volume is not more than about 0·2 ml. (usually much less), it follows that the total potassium content of the blood

Table 1. *By flame photometer*

onic concentrations expressed as m.equiv. (mM.)/l. The estimated errors in the columns refer to flame photometer surements only. No estimate of the sampling error (volume measurement) was made, but may be taken as not more 5 % in each case.)

imal	Adult				Solitary 5th instar				Gregarious 5th instar			
	Grass fed		Starved		Grass fed		Starved		Grass fed		Starved	
	Na	K	Na	K	Na	K	Na	K	Na	K	Na	K
1	95±4	13±1	85±3	11±1	82±3	23±2	140±5	17±1	75±3	30±2	122±4	9±1
2	95±4	14±1	73±3	14±1	82±3	30±2	107±4	25±2	95±4	35±3	86±3	16±1
3	105±4	19±1	156±5	11±1	85±3	23±2	97±4	17±1	104±4	33±3	140±5	11±1
4	98±4	16±1	97±4	14±1	72±3	43±3	117±4	10±1	79±3	27±2	116±4	11±1
5	100±4	20±1	102±4	10±1	79±3	27±3	114±4	13±1	88±3	33±3	112±4	13±1
6	100±4	25±2	105±4	16±1	83±3	37±3	106±4	11±1	92±4	20±1	83±3	20±1
7	127±5	29±2	—	—	85±3	27±2	134±5	11±1	116±4	32±2	100±4	9±1
8	64±3	17±1	—	—	—	—	120±4	25±2	108±4	33±3	—	—
9	—	—	—	—	—	—	95±4	13±1	130±5	28±2	—	—
10	—	—	—	—	—	—	85±3	10±1	87±3	18±1	—	—
11	105±4	19±1	100±4	9±1	108±4	15±1	145±5	22±1	—	—	—	—
12	118±4	17±1	100±4	9±1	129±5	42±3	83±3	10±1	—	—	—	—
13	154±5	25±2	96±4	7±1	116±4	28±2	104±4	12±1	—	—	—	—
14	129±5	16±1	109±4	11±1	87±3	25±2	94±4	11±1	—	—	—	—
15	108±4	16±1	104±4	9±1	70±3	40±3	92±4	12±1	—	—	—	—
16	80±3	20±1	—	—	72±3	35±3	108±4	11±1	—	—	—	—
17	110±4	12±1	—	—	104±4	40±3	87±3	12±1	—	—	—	—
18	80±3	14±1	—	—	112±4	19±1	100±4	10±1	—	—	—	—
19	—	—	—	—	—	—	92±4	9±1	—	—	—	—
20	—	—	—	—	—	—	130±5	11±1	—	—	—	—
Av.	109	18	103	11	91	30	109	14	97	29	108	13
.D.	21·9	4·8	20·4	2·7	16·8	8·3	20·1	4·9	17·1	5·8	20·1	4·0
.E.	±5·7	±1·2	±6·5	±0·8	±4·5	±2·1	±4·6	±1·1	±5·7	±1·9	±8·2	±1·6
	Potassium. S.E. of difference between means, 2·5. $t=4·4$. Significant. *Sodium.* S.E. of difference between means, 8·7. $t=0·7$. Not significant				*Potassium.* S.E. of difference between means, 2·3. $t=7·1$. Significant. *Sodium.* S.E. of difference between means, 6·6. $t=2·7$. Significant.				*Potassium.* S.E. of difference between means, 2·7. $t=5·4$. Significant. *Sodium.* S.E. of difference between means, 9·7. $t=0·9$. Not significant			

is about 0·16 mg. or less (assuming 20 mM. K/l.) and hence the animals take in more than 20 times their total existing blood potassium content every 24 hr. As a large part of this probably passes from the gut into the blood, the potassium load for excretion imposed on the malpighian tubules must be great if the level in the blood is to be kept down.

Even if insects were highly efficient at excreting potassium and could do so at a fast rate, there would be a tendency for the blood potassium to rise during continuous feeding. The bug *Rhodnius* has been shown by Ramsay (1952) to be capable of excreting potassium efficiently, but rather slowly, so that the addition of extra potassium to its normal diet, which does not contain large amounts, resulted in a large increase in blood potassium. It is not surprising, therefore, that among feeding locusts some quite high potassium contents are found (Table 1) or that the content is appreciably lower during starvation, when potassium is still being excreted although no more is being taken in. The values obtained following a short period of starvation may be taken as the 'norm' and the high values as variations imposed by the diet. The higher average value for potassium found in feeding hoppers is no doubt due to their greater food intake.

MUSCLE MEMBRANE POTENTIAL

In the earlier paper (Hoyle, 1953*a*) a curve was presented showing the relation between the resting potential (R.P.) difference across the locust muscle membrane and the concentration of externally applied potassium. Thus if the resting potential can be determined whilst the fibres are bathed in the animal's own blood, it should be possible to determine the amount of freely ionized potassium in the blood indirectly from this curve. This would therefore be a purely physiological indirect method which might be used to confirm direct chemical measurements of blood potassium. Resting potentials of muscle fibres bathed in blood can be obtained with intracellular electrodes. A small area of cuticle is removed from a leg which has

Table 2. *By resting potential*

(Ionic concentrations expressed as m.equiv. (mM.)/l. Adult locusts. Potassium calculated from R.P./K. curve.)

Animal	Grass fed		Starved	
	Av. R.P. (mV.)	K	Av. R.P. (mV.)	K
1	36 (30–40)	30	56 (50–60)	12
2	35 (20–50)	31	62 (60–65)	9
3	60 (60–60)	10	50 (50–50)	16
4	33 (30–35)	35	58 (50–70)	11
5	55 (40–60)	13	60 (50–70)	10
6	42 (40–60)	23	63 (60–70)	9
7	48 (40–60)	17	50 (50–50)	16
8	50 (40–60)	16	52 (40–70)	14
9	47 (40–60)	18	55 (50–60)	13
10	43 (40–60)	22	60 (50–70)	10
11	50 (40–60)	16	61 (50–70)	10
12	47 (40–60)	18	60 (50–70)	10
13	44 (40–60)	22	—	—
14	47 (40–60)	18	—	—
Av.	45	20	57	12
S.D.	7·5	—	4·7	—
S.E.	±2·0	—	±1·4	—

S.E. of difference between means, 2·5. $t=4·8$. Significant.

been freshly isolated or is still attached to the body, and another part of the same leg earthed. A microelectrode is then thrust into fibres of the exposed muscle, still bathed in blood, and the average resting potential determined. The zero potential line is not quite so easy to establish as in the normal technique for intracellular recording when the muscle is surrounded by a large volume of earthed saline, but provided the animal is not too dry no serious difficulty is experienced. A set of such measurements is shown in Table 2. The range of values is also illustrated in Fig. 2 where they are shown plotted on the previously-determined R.P./K curve, and in Fig. 3 where they are compared with other muscle properties and blood concentrations. Although it has not been convenient to make resting potential and flame photometric determinations on the same animal, the ranges of values for blood potassium obtained from different groups of similar animals using the two methods are in striking agreement, and show the value of the indirect method. They clearly show that the muscle fibre resting potential may decrease considerably *in situ* during feeding, and that this is most probably caused by an increase in freely ionized potassium in the haemolymph.

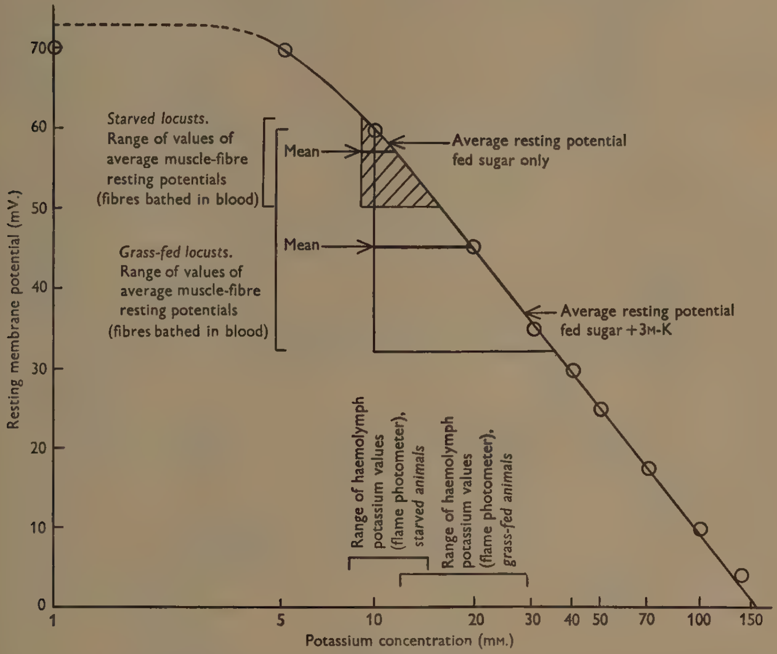

Fig. 2. Resting potential/potassium curve on which are also plotted the range of values of resting potential obtained by measuring in freshly exposed muscle fibres still bathed in their own blood. The range of values for flame-photometrically determined potassium is also shown.

The high value of blood potassium during feeding places the resting potential on the steeply sloping part of the R.P./K curve. This is a unique situation. Vertebrate, crustacean and molluscan bloods have a potassium content such that the muscle resting potentials are near their maximum possible value. Normal fluctuations in blood-potassium concentration of these groups never (as far as is known) attain a high enough value to exert a physiological effect. This is clearly not the case with *Locusta*. Further, the drop in blood potassium during the initial stages of starvation is great enough to raise the R.P. by about 12 mV. This means that following nerve stimulation a greater muscle action potential will be produced in the partially starving than in the feeding animal, and in the perfused leg this is demonstrably associated with a greater degree of contraction. Hence it may be possible to show that the locust muscles contract more vigorously after a few hours without food.

MUSCLE TENSION

Measurements have been made of muscle tension developed by the extensor tibialis muscle of the metathoracic (jumping) legs of 5th-instar hoppers following nerve stimulation. Animals were taken from stock, reared crowded and the lengths and thicknesses of the tibiae measured. Females, with their slightly larger overall dimensions, were used most frequently. Animals were then sorted into pairs having similar leg measurements and marked with paint for subsequent identification. One member of each pair was then deprived of food for a few hours, after which the animals were mixed together again and immediately tested. The marking was carried out by Dr Ellis so that the author did not know at the time of measuring tension which animals had been deprived of food.

After the jumps had been measured (see below) the hoppers were immobilized by pressing on to soft plasticine, the distal end of the tibia connected to an electromechanical transducer and the flexor tibialis muscle denervated. A single electrical stimulus (above a sufficient intensity) applied to the crural nerve produces an all-or-nothing twitch of the extensor tibialis. This tension was measured on all the animals, as was also the considerably greater twitch tension (due to mechanical summation) produced in response to a close pair of stimuli (cf. Fig. 1). Tetanus tension, which is very much greater than twitch tension, was measured only on a few animals, but was always small when twitch tension was small. After only 6–7 hr. of starvation the range of twitch tension was found to have shifted upwards by about 30% (Fig. 3).

JUMPING

The 'fast-fibre' twitch mechanism of the extensor tibialis muscle is used by the animals to produce hopping and jumping movements. Hopping is essentially a means of linear progression encountered occasionally or repetitively during normal marching locomotion by immature locusts. There is no arrest in linear progression before a hop, both hind-legs are simply extended synchronously and rapidly, with appropriate adjustments in the first pair of legs, thus achieving an upward and forward movement. In jumping, which is an escape mechanism, any linear motion is first arrested, the tibia is flexed tightly against the femur and the swing of the latter is then effected from this position. A jump uses the maximum available leverage and gives both a higher and longer displacement than a marching hop. The jump can be elicited by tapping the ground immediately behind a resting hopper. In this way a natural movement of an intact animal, the magnitude of which must depend upon the tension developed by the extensor tibialis muscles, can be determined, it being an easy matter to measure the distance jumped.

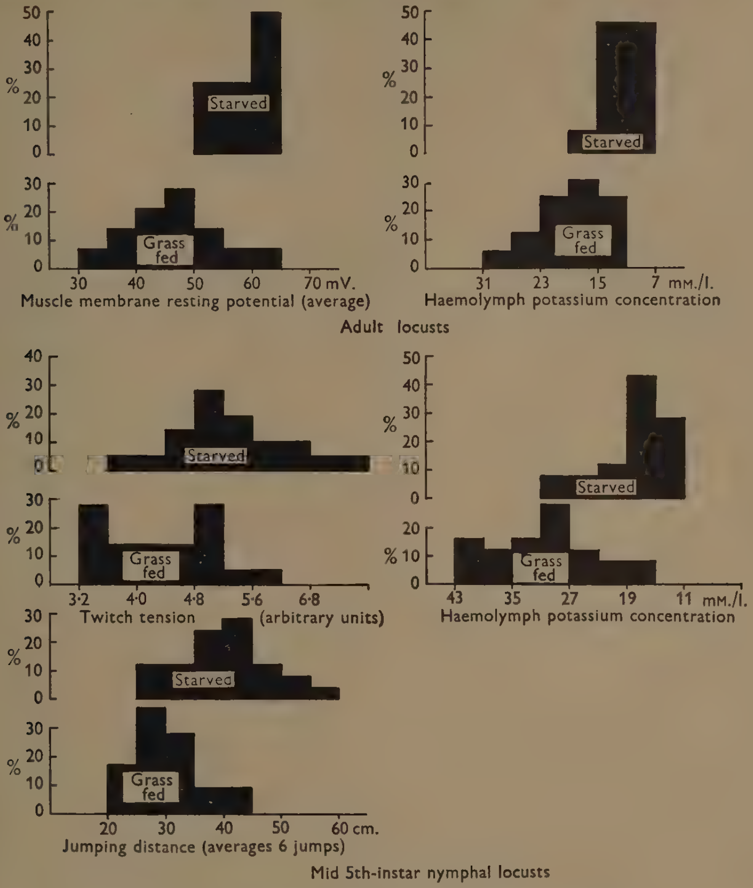

Fig. 3. Histograms of certain values to show the shift during starvation. Jump distances and twitch tensions from about sixty animals in each case.

Observations were made upon all the animals used for the determination of the effect of feeding on twitch tension, as well as upon others subjected to the same preliminary treatment. Each animal was made to jump at least six times and the jumps averaged. Jumps of less than 20 cm. were regarded as being in the nature of hops only and ignored. The results are illustrated in Fig. 3. There is a shift in the range of distance jumped following starvation and, as in the case of twitch tension, the increase is about 30 %. The change in distance jumped need not be due entirely to the change in muscle tension; the slight extra weight of the full abdomen might reduce the height of the jump, and possible inhibitory effects of a full gut on central activity cannot be ruled out. However, the extent of the observed shift in distance jumped is just what one would expect if the changed peripheral effectiveness were alone responsible.

ARTIFICIAL DIETS

The above results show fairly clearly that high blood-potassium concentrations in *Locusta* are a direct consequence of high diet potassium, but it was realized that feeding on artificial diets containing known potassium concentrations was needed in order to strengthen the case. Only preliminary steps have been made in this direction, and an ideal artificial diet has certainly not been found. The locusts will, however, eat small squares of filter-paper which have been soaked in strong glucose, or preferably sucrose, solution. Squares of 2 × 2 cm. are provided and the total area eaten in a given time ascertained. Fifth-instar hoppers eat an average of 124 mm.^{2} of no. 1 Whatman paper soaked in molar sucrose in 12 hr. If potassium chloride solution is added to the squares an appreciable area of paper is still eaten. The potassium content of the squares can quickly be determined by extracting the salt with distilled water and titrating for chloride, subsequently transposing to potassium values. Hence the total potassium consumed by a given animal can be determined. It was found that animals fed on sugar and filter-paper alone had muscle resting potentials (Fig. 2) and twitch tensions similar to those of starved animals. Animals eating in 12 hr. an average of 50 mm.^{2} paper soaked in both sucrose and 3 M-K (representing an intake of 0·8 mg. K, or the equivalent of a grass meal of 9 cm.^{2}) had a greatly reduced twitch tension, a blood content of about 35 mM K/l., and a resting potential of only 35 mV. The animals fed on the filter-paper plus potassium plus sucrose had almost no blood in them and out of twenty animals thus fed only one yielded sufficient blood for analysis. This may indicate that extra water was lost from the blood of these animals during the attempt to excrete the excess potassium. The very high value of the blood potassium in animals fed on filter-paper plus sugar plus 3 M-K, in spite of a small total potassium intake as compared with grass-fed animals suggests that the water obtained from the grass and/or by drinking is essential for efficient potassium excretion.

DISCUSSION

It has long been known that potassium ions in abnormal concentrations affect several properties of muscular activity, including the characteristics of excitation, contraction, metabolism (see Sandow & Kahn, 1952). These observations were made in studies aimed at yielding fundamental information regarding the various processes involved in muscular contraction. In most animals, as was mentioned earlier, blood potassium does not vary sufficiently in concentration to bring about physiological changes of the type studied in these investigations. In certain insects, however, where the normal blood-potassium level is very high, it may be safely stated that some properties of muscle activity will, in fact, be affected, provided that the muscle fibres function in a similar way to those studied in *Locusta*. Thus an insect with perpetually high potassium may be condemned to sluggish movement. This is not necessarily biologically disadvantageous. In fact, many herbivorous insects are remarkably sluggish in movement, especially in the larval stages, a factor which gives them a measure of protection against observation by predators. The situation has another important compensation. When the food supply becomes scarce there will be a decrease in blood potassium which will change the muscle properties in the direction of greater overall speed and power. Hence a valuable aid to emigration from the exhausted region is automatically supplied. On finding a new region of suitable food material an inevitable quietening down of activity must follow.

In the foregoing investigations it has been shown that locust blood may contain up to 30 mM. K/l. in the adult stage, up to 40 mM. K/l. in nymphs. These are high values compared with vertebrate blood, but are not as high as in other herbivorous insects (table in Roeder, ed., 1953), nor are they as high as had been expected. Boné (1944) claimed that the blood Na/K ratio is less than unity in herbivorous insects, and at the outset of the experiments described in this paper the author regarded the locust as a typical herbivorous insect on the grounds of its habitual grass diet. In fact, the locust blood Na/K ratio is very much greater than unity, and therefore *Locusta* must be regarded either as an omnivore (it will eat almost anything when starved), or as a serious exception to Boné's rule. In the locust there is clear evidence that the potassium concentration in the blood varies enormously from one individual to another when the animals are kept in the presence of suitable food (Table 1). When the food is removed, the potassium concentration is not only reduced, but it tends to a more uniform value (Table 1). The resting level in the blood when there is no further excretion of potassium is probably about 10 mM./l. Most of the cation content of the blood is made up of sodium ions at about 110 mM./l.

Locusts feed irregularly and intermittently (Ellis, 1951), so although food is continually present it cannot be assumed that animals have been feeding very recently. There is probably a continual fluctuation in the level of blood potassium and random selection of animals in different phases of feeding might account for the large individual variation.

The tonus of the muscles of *Locusta* is maintained by background discharges of impulses down the 'slow' fibres (Hoyle, 1953*b*, *c*). Superimposed on this flow are occasional impulses down the fast fibres and short bursts of higher frequency slow fibre activity. These are responsible for spontaneous and reflex movements. The proprioceptive feed-back into the central nervous system helps to maintain the level of both tonic and active contraction. A decrease in the capacity of the muscle to contract to a given nerve stimulation, such as must follow the raising of the blood-potassium level, will lead to reduced feed-back and finally, to decreased tonus and spontaneous activity. It may be expected, therefore, that the pattern of behaviour will itself be affected by starvation not only by such factors as reduced blood sugar and empty gut, but by a mechanism which is dependent on the physiological state of the muscle fibres, the effector organs of behaviour themselves.

SUMMARY

1. The concentrations of sodium and potassium in the haemolymph of the locust *Locusta migratoria migratorioides* R. & F. have been determined by flame photometry. The molar ratio of sodium to potassium is normally about 5 : 1.

2. During a short period of starvation the potassium content of the haemolymph decreases by as much as 50%.

3. The change in potassium is adequate to account for observed differences in muscle membrane resting potentials and for variations in the mechanical responses of the muscles to nerve stimulation.

4. The ability of 5th-instar hoppers to respond to stimulation by hopping or jumping is increased after a short period of starvation owing to the increased mechanical responses of the muscles.

5. It is suggested that changes in diet potassium will be found to influence behaviour of locusts by the direct effect of the potassium on muscle fibre activity.

The author wishes to thank Prof. H. Nicol of the Scottish Agricultural College, who provided the data on the potassium content of leaves of *Agropyron repens*, and Dr P. E. Ellis for her willing help at all stages of the work. The electrical measurements were made with apparatus purchased with a Royal Society grant-in-aid award. The experimental animals were supplied by the Anti-Locust Research Centre.

REFERENCES

Boné, G. J. (1944). Le rapport Na : K dans le liquide coelomique des insects. *Ann. Soc. zool. Belg.* **75**, 123–32.

del Castillo, J., Hoyle, G. & Machne, X. (1953). Neuromuscular transmission in a locust. *J. Physiol.* **121**, 539–47.

Ellis, P. E. (1951). The marching behaviour of hoppers of the African migratory locust in the laboratory. *Anti-Locust Bull.* **7**, 1–48.

Ellis, P. E. & Hoyle, G. (1954). A physiological interpretation of the marching of hoppers of the African migratory locust (*Locusta migratoria migratorioides* R. & F.). *J. Exp. Biol.* **31**, 271.

Hodgkin, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339–409.

Hoyle, G. (1953*a*). Potassium ions and insect nerve muscle. *J. Exp. Biol.* **30**, 121–35.

Hoyle, G. (1953*b*). 'Slow' and 'fast' nerve fibres in locusts. *Nature, Lond.*, **172**, 165–7.

Hoyle, G. (1953*c*). Intracellular recording of 'slow' and 'fast' fibre activity from an insect muscle. *J. Physiol.* **121**, 32*P*.

Ramsay, J. A. (1952). The excretion of sodium and potassium by the malpighian tubules of *Rhodnius*. *J. Exp. Biol.* **29**, 110–26.

Roeder, K. D. (1953). Editor. *Insect Physiology*, pp. 1100. Wiley. New York.

Sandow, A. & Kahn, A. J. (1952). The immediate effects of potassium on responses of skeletal muscle. *J. Cell. Comp. Physiol.* **40**, 89–114.

A PHYSIOLOGICAL INTERPRETATION OF THE MARCHING OF HOPPERS OF THE AFRICAN MIGRATORY LOCUST (*LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.)

By PEGGY E. ELLIS and G. HOYLE

Departments of Zoology, University College, London, and the University of Glasgow

(*Received* 17 *September* 1953)

INTRODUCTION

Under certain conditions laboratory-reared hoppers (nymphs) of the locust *Locusta migratoria migratorioides* (R. & F.) show a characteristic behaviour pattern which is similar to the marching of hopper bands in the field. During marching, groups of individuals walk and hop continually in the same direction for long periods, the body being held with the abdomen raised well off the ground, the antennae erect and the frons perpendicular to the long axis of the thorax. Hoppers will circumambulate small cages provided a light is placed centrally above them and certain other conditions are satisfied (Ellis, 1951). In all instars the most vigorous and prolonged marching takes place at temperatures between 29 and 35° C. To get good marching, hoppers should not be near to a moult, must have been reared crowded for 7–9 days previous to a test and at least twenty individuals should be placed together in the cage.

When grass is present, marching is also dependent on increased radiant heat from the overhead light and on a current of warm air. Although some individuals march from the beginning of such tests, maximal activity is not reached for 2–3 hr., when about 40 % of the time is spent in marching. Grass, radiant heat (from the sun) and currents of warm air are a normal part of the environment in the field, so considerable interest resides in field observations on the maximal time spent marching by wild hoppers. Recent observations by one of us (P.E.E.) on wild hoppers of another species (*Schistocerca gregaria* Forsk) marching through dense bush in the Ogaden, Ethiopia, showed an average maximal time spent marching of only 28 %, whilst the highest ever recorded was only 40 %.

By contrast, very vigorous marching with maxima of 60–70 % or more time spent marching is commonly obtained in the laboratory in foodless cages even without increased radiant heat and draught. In foodless cages the formerly well-fed hoppers spend the first 15–20 min. of the test period resting in the postprandial posture, with the abdomen on the ground and the hind-legs folded close to the body. They gradually acquire levator-muscle tonus, and the abdomen is progressively raised until marching posture is apparent. At the same time spontaneous movements become evident and eventually individuals start to potter round the cage. The pottering is interrupted by frequent rests, but these become fewer as speed increases and as more and more individual hoppers leave the resting position to take part in the activity. From pottering, marching soon emerges, a few individuals at a time gathering impetus and 'drive'; the resting periods become further curtailed. The increases in both marching speed and percentage time spent marching present smooth curves of exponential type when plotted against time (Ellis, 1951), the maximum being achieved 3–5 hr. after transfer to the foodless cage. Once initiated this group activity is maintained at the high level for many hours, and in one test carried out at 32° C. marching only began to decline after the hoppers had been starved for 12 hr., probably as the result of approaching exhaustion. It would be very interesting to know whether wild hoppers are ever sufficiently starved to show this very vigorous marching; unfortunately, no data are available on this point.

It is the purpose of the present paper to consider further the very vigorous marching which results from starvation in the light of the recent observation (Hoyle, 1954) that the properties of the neuro-muscular apparatus of *Locusta* hoppers change during the early stages of starvation in such a way as to render the muscles capable of a greater contraction following similar nerve discharge. These changes in the mechanical properties of the muscles are associated with a fall in the level of the blood potassium which results in a raising of the muscle resting potential, thereby rendering the muscle capable of giving larger muscle action potentials and greater twitch tension. As hoppers normally obtain rich supplies of potassium salts in their food (mainly grass) and potassium is actively excreted (cf. Ramsay, 1953), starvation naturally results in a reduction of the blood potassium. It is possible, therefore, that the potassium changes are concerned with the development of the vigorous marching pattern in starving hoppers. This idea could be stated in the following way, namely that a high blood potassium content reduces marching activity under otherwise optimal conditions. The hypothesis was tested by feeding hoppers under crowded conditions on a diet containing adequate soluble carbohydrate for energy requirements, together with various known amounts of a simple potassium salt. If reduced blood potassium is responsible for the very greatly increased marching of starved individuals, then hoppers fed on sugar plus adequate potassium should behave in a similar way to grass-fed animals, whereas hoppers fed on sugar only should show the same marching activity, quantitatively, as starving animals.

EXPERIMENTAL METHOD

Locusta hoppers will eat filter-paper soaked in sugar (sucrose is preferred) solution (Hoyle, 1954). Although the area eaten in a given time is considerably less than the area of grass which would be eaten in the same time, this nevertheless affords a simple method of ensuring feeding for a few hours on carbohydrate (sufficient for energy requirements) and on a potassium salt in any desired concentration. Hoppers were marked with spots of poster paint so that they could be identified individually, divided into groups and fed in different ways overnight. Some hoppers were starved, some fed on filter-paper soaked in sugar, some on sugar plus potassium chloride solutions of different known concentrations and a final group on grass. Next morning all the hoppers were placed together in a cage 2 × 2 × 1 ft. high which was provided with a central, overhead light but contained neither food nor water. The tests were carried out in a constant temperature room maintained at 30° C., excessive heating being prevented by placing a water screen between the cage top and the lamp. During 10 min. periods, at intervals of half an hour, the activities of each marked hopper were noted in turn, and from the readings the percentage time spent marching was calculated and plotted as percentage marching against time. Tests lasted for 6 hr., and the complete experiment was repeated several times with each of three different potassium concentrations as the only variable.

RESULTS

The average percentages of time spent marching during the tests are shown in Fig. 1 *a*, *b*. Hoppers deprived of food all night, those fed on M. sucrose soaked filter-paper and those fed on M. sucrose plus M. KCl all behaved in a quantitatively similar way. None of them showed the postprandial resting posture and marching soon began, increased rapidly in occurrence to a maximum of about 60 % after only 2 hr. At first, hoppers fed on grass showed the postprandial posture and came on to march only slowly, taking at least 4 hr. to achieve the same maximum of 60 % marching, in spite of the presence of several fully marching starved or sugar-fed hoppers. For the first hour hoppers fed on 2 M. K plus M. sucrose behaved in a similar way to hoppers fed on sugar only, i.e. some of them had no postprandial posture and soon started to march. Others, however, continued to rest so that after 1 hr. a delay was apparent compared with starving hoppers. After 4 hr. the common maximum had been reached. These hoppers survived unaffected by their peculiar diet and the general similarity between a few of them and grass-fed ones was notable. The 3 M. K forms never reached the higher maxima and actually started to decline before the test period elapsed, so that after 6 hr. they were marching for only 30 % of the time. Some died a few days after the test.

The graphs (Fig. 1) show that the special diets containing potassium retarded marching activity. The initial activity of these groups, when treated as a whole, did however resemble the starved type rather than the grass-fed type. This may be due to an inhibitory effect of a full gut affecting the latter but not the former, for the gut of grass-fed animals is always distended with food, whereas that of the filter-paper ones is only partially filled (Table 1). However, one or two animals 'dodging the issue' and eating only minute quantities of the potassium-laden paper, as is suggested by the individual results shown in Table 1, could produce an initial distortion of what might otherwise be a full retardation, in a homogeneous population of animals which had taken their fill of potassium laden paper. The failure of the 3 M. K-fed hoppers to reach the activity shown by the other groups suggests that many of them had consumed far more potassium salt than they could excrete in the time available. The absence of drinking water for 20 hr. no doubt reduces their capacity for excreting salts.

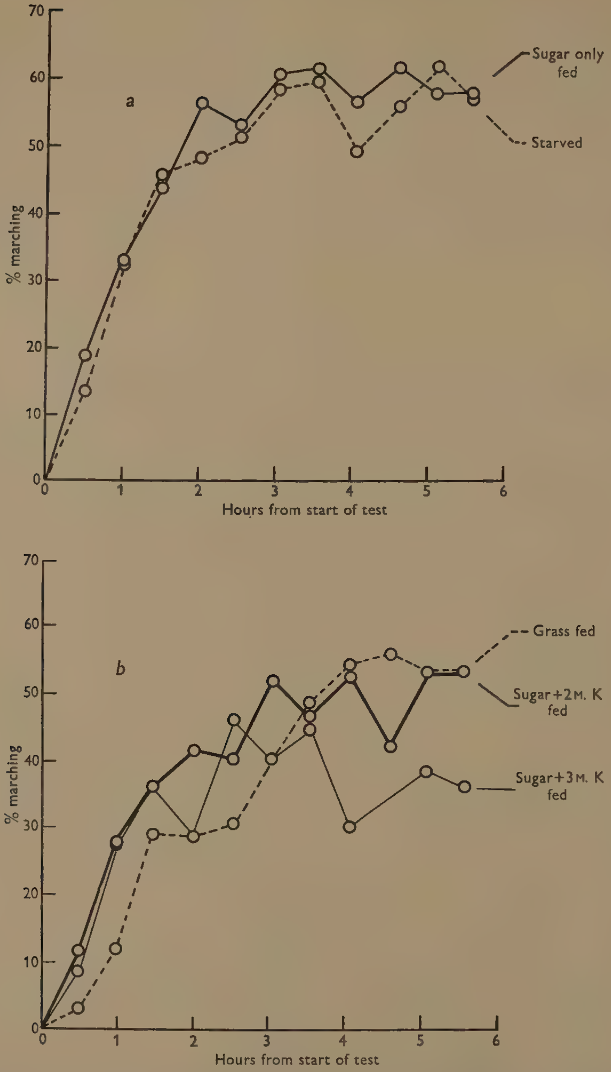

Fig. 1. The percentage marching by hoppers plotted against time following transfer to a test cage after feeding for 20 hr. on various diets. The curves are averages for two to nine runs each. (*a*) Unbroken line: starved; broken line: fed on sugar and filter paper only. (*b*) Broken line: grass-fed; thick line: fed on 2 M. K + sucrose; thin line: fed on 3 M. K + sucrose.

Table 1. *The quantities of food in the alimentary tracts of hoppers fed on special diets for 20 h.*

(3 = distended, 2 = full, 1 = some, 0 = empty.)

No. of hopper	Grass-fed			Fed on sugar + 2 M. K			Fed on sugar only			Starved		
	Crop	Mid-gut	Hind-gut-	Crop	Mid-gut	Hind-gut-	Crop	Mid-gut	Hind-gut	Crop	Mid-gut	Hind-gut
1	3	3	3	2	1	0	3	1	1	1	1	1
2	3	2	2	2	1	0	1	0	0	1	1	1
3	3	1	0	2	1	0	3	2	1	1	1	0
4	2	1	1	0	1	1	1	1	0	0	0	0
5	3	2	1	1	1	1	1	1	1	1	1	0
6	2	2	1	1	0	0	1	2	1	1	1	1
7	3	2	2	1	1	1	1	1	2	0	0	0
8	3	2	2	1	2	1	1	1	1	1	0	1
9	3	2	1	2	1	0	1	1	0	1	1	1
10	3	2	2	2	1	1	1	1	1	1	0	0
11	3	3	3	2	0	1	1	1	0	1	1	1
12	3	1	0	1	1	0	1	1	2	1	1	1
13	3	3	2	1	1	0	1	2	0	1	0	0
14	3	3	2	1	1	1	2	1	1	0	0	1
15	3	2	2	2	1	1	1	1	1	1	0	0
Av.	2·9	2·1	1·6	1·4	1·0	0·5	1·3	1·1	0·8	0·8	0·5	0·5

The results strongly suggest that the changing potassium concentration in the blood has an effect on the behaviour of hoppers. They further indicate that the physiological consequences of the decreasing potassium concentration may play an important part in building up the excitatory state which manifests itself in marching.

DISCUSSION

An assessment of the role of changing potassium ion concentration and other factors in the building up of the excitatory state leading to marching seems desirable. The factors may conveniently be divided into two groups: environmental factors acting externally on sense organs of all kinds, and internal factors affecting the properties of the neuromuscular apparatus. The work of Ellis (1951, 1953) has thrown much light on the external factors. Most of them are in the nature of 'limiting factors', i.e. each must have a value within certain fairly rigid upper and lower limits before the final activity can be manifested normally. Within these limits each is stimulatory. They include temperature, radiant heat, air velocity, optic stimuli and contacts with other locusts. Marching is shown to be a group activity by its almost complete dependence on the last two factors. Hoppers reared in isolation for 7–9 days show greatly reduced marching activity when tested in the normal way. This may well be due to some internal change in the animal, other than a change in blood potassium concentration, brought about by the rearing conditions. Of internal factors only the changing potassium concentration of hoppers reared crowded has so far been fully studied. Although this could affect nerve conduction, central synaptic and neuromuscular transmission as well as muscle-fibre properties, it is thought that its effect is principally confined to the last two for evidence has been given previously (Hoyle, 1952, 1953) that the nervous system is surrounded by a sheath which protects the axons at least, and probably the whole nervous system from fluctuations of blood potassium content. The principal effect of the fluctuations would therefore be to render the muscular contractions following nerve stimulation greater or lesser according to the potassium level. In other words, the extent to which the motor nerve output from a given nerve centre successfully produces activity must depend ultimately on the blood composition, and motor activity may be expected to be abnormal just before or just after moulting and during heavy feeding, on the grounds of changing peripheral effectiveness alone.

Among the external factors at least two, contactual and optic stimuli, act as links in positive feed-back systems when hoppers are in groups. Each leads to activity which in turn promotes a further increase in itself. There is no negative feed-back, for the motor activity produced does not oppose any of the initial stimuli. Thus the movement of a locust provides optic, contact and perhaps also vibrational stimulation of its neighbours which are themselves stimulated to move, thus providing new sources of activation for the first and so on. The overall effect is of a 'snowball' type and this is a most effective analogy when one is familiar with the laboratory experiments. The problem of who starts the ball rolling is not a difficult one. Arthropod nervous systems are well known to exhibit spontaneous discharges (Prosser, 1934; Roeder, 1948) and this kind of activity is probably responsible for maintaining tone in the muscles. It may also produce random active muscular activity. These small random movements must be supposed to lead to temporary changes in the nervous centres, so that an excitatory state of relatively long persistence is progressively built up in them. As long as the state persists two important consequences ensue: spontaneous activity increases and synaptic transmission is facilitated. Although these are still hypotheses they are reasonable speculations and are open to experimental test. Grass-fed hoppers exhibit little tone and almost no random movements (visual and cinematographic records), starved ones show considerable tone and fairly frequent random movements. These differences could be accounted for by the differences in muscle response to the background spontaneous activity which must follow the potassium ion changes. When the scene is set for marching to appear and the 'snowball' commences its roll then the initially starved animals will be at a distinct advantage because their muscle system is at this time a more effective instrument than that of recently fed animals; they are the first to move actively, the first to potter, and the first to achieve the excited state which results in marching. There is no evidence that marching can appear in any other way than through this snowball phenomenon which takes a minimum of 2 hr. to complete. Recent observations by Stower (1953) and Ellis (unpublished) make it clear that a similar process must occur in the field before marching commences.

The marching activity of hoppers is not the property of a particular instar, hoppers of any instar will march under the same conditions. Further, this activity seems in some respects to be the counterpart of adult migratory activity, which also requires an initial period of mutual stimulation (Kennedy, 1951). Both are long-continued, restless movements without any specific orientation or goal. These facts suggest the existence of an inherited nerve centre or centres common to all instars and adults, which can give rise at the motor level to marching (or 'migratory') activity when adequately stimulated.

Marching appears both in the laboratory and in the field in the presence of grass so it cannot be a 'drive' in response to the absence of food. A simple mechanistic interpretation has been sought which takes into account the marching in the presence of food, which explains the progressive 'snowball' build-up of the activity and its somewhat rigid, unadaptable expression as laboratory marching, and which also incorporates the internal effects of changing potassium. A scheme which appears to satisfy these requirements in the present state of knowledge is presented above. The large square in Fig. 2 represents the nerve centres concerned with the marching activity. External factors which stimulate the activity, probably through excitatory or facilitatory action at synapses within the centres are represented by solid arrows feeding into the centres. The hollow arrow represents the appropriate motor nerve output which produces locomotion (pottering and marching). The response to this output given by the muscle units will depend on the immediate composition of the haemolymph, provision for this is made by placing 'blood' as a bridge across the gap between nerve activity and expression by the muscles.

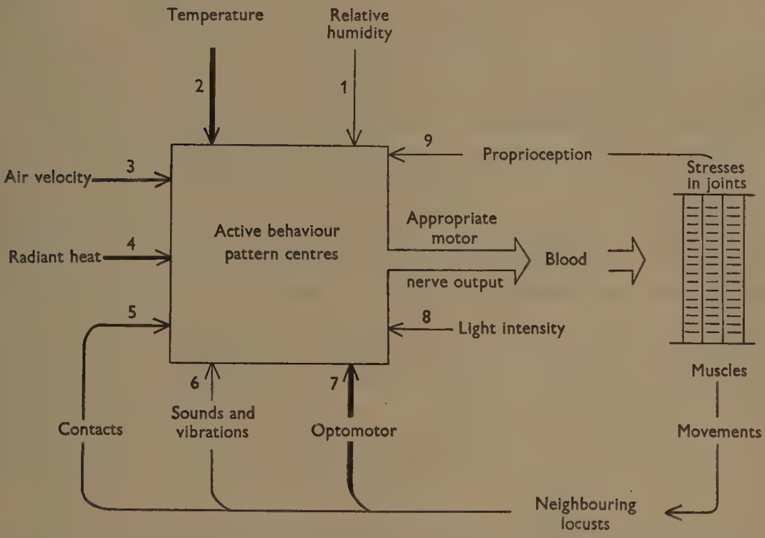

Fig. 2. Scheme illustrating the interpretation of marching behaviour. Relative importance of environomental factors is illustrated by thickness of arrows. For explanation see text.

Three of the stimulatory channels (5–7) are regenerative (positive feed-back) forming closed loops which make the appropriate activity self-re-excitatory. Proprioception may on balance be stimulatory, but channels 5, 6 and 7 are only complete in the presence of other locust hoppers.

According to this scheme there is no reason why an individual isolated from its neighbours should never march as this would depend on quantitative considerations involving the various stimulatory channels. Laboratory tests do in fact show that one hopper by itself will sometimes march but only for short periods (Ellis, 1953). The stimulatory factors act independently to a considerable extent and summate, so that they are also compensatory. Thus an increase in air velocity would be expected to produce activity when radiant heat is low and vice versa. The independence and compensatory nature of the factors probably accounts for the production of some marching in the presence of food when the gap between nerve activity and muscle contraction is not perfectly bridged owing to high blood potassium. Under these conditions the increased stimulation by air currents and radiant heat, by acting as additional stimulations, compensate to some extent for the poorer state of the muscles. Under no circumstances can the excitatory level in the motor centres build up other than slowly, probably by quantal summation of slowly decaying effects of afferent stimuli. Once built up it decays equally slowly. An immediate temporary inhibition of some part of the neuromotor system is possible as occurs after a tap on the cage whilst marching is in progress, when all marching suddenly ceases and each animal becomes motionless. The inhibition may occur at central synapses before the final motor pathways or possibly peripherally (Ripley & Ewer, 1951), but it does not destroy the excited state, for a moment after the arrest of activity marching is resumed at about the same level as before the tap.

Marching activity is in essence the normal state for hoppers of the gregarious phase under the prescribed conditions, a conclusion similar to that arrived at by Kennedy (1951) in regard to the 'migratory' activity of adult *Schistocerca*. It is likewise a gregarious phenomenon, for although the tendency to march or 'migrate' is inherent, the specific associated behaviour pattern is only elicited by mutual stimulation. Its expression is conditioned by internal physiological conditions, one of which is in direct relationship with the environment, i.e. the presence or absence of abundant potassium-rich vegetation, but if all factors are favourable marching must appear just as surely as withdrawal of the hand must follow a sharp prick of the finger in man.

SUMMARY

1. Hoppers of the locust *Locusta migratoria migratorioides* R. & F., which have been reared crowded, exhibit characteristic marching behaviour in the laboratory in foodless cages under standard conditions.

2. The rate of attainment of maximum marching by a given group of hoppers is greatest following a short period of starvation immediately preceding transfer to these conditions.

3. A meal of filter-paper soaked in sugar solution only, exerts no retarding effect

on the rate of attainment of full marching, whilst if an adequate concentration of potassium salt is added in addition a definite retardation is observed.

4. In locusts actively feeding on grass the potassium content of the blood is relatively high, and it is suggested that this causes a reduced muscular efficiency which may explain retardation in attainment of full marching.

5. The marching pattern is the result of the activity of particular nerve centres which require a period of activation by a combination of factors before full expression is achieved. Two final factors are essential for the maintenance of the marching state, a low concentration of blood potassium and mutual stimulation by other marching hoppers.

REFERENCES

Ellis, P. E. (1951). The marching behaviour of hoppers of the African migratory locust (*Locusta migratoria migratorioides* R & F) in the laboratory. *Anti-Locust Bull.* 7.

Ellis, P. E. (1953). The gregarious behaviour of marching *Locusta migratoria migratorioides* (R. & F.) hoppers. *J. Exp. Biol.* **30**, 214–35.

Hoyle, G. (1952). High blood potassium in insects in relation to nerve conduction. *Nature, Lond.*, **169**, 281–2.

Hoyle, G. (1953). Potassium ions and insect nerve muscle. *J. Exp. Biol.* **30**, 121–35.

Hoyle, G. (1954). Changes in the blood potassium concentration of the African migratory locust (*Locusta migratoria migratorioides* R. & F.) during food deprivation and the effect on neuromuscular activity. *J. Exp. Biol.* **31**, 260.

Kennedy, J. S. (1951). The migration of the desert locust (*Schistocerca gregaria* Forsk.). *Phil. Trans.* B, **235**, 163–290.

Prosser, C. L. (1934). Action potentials in the nervous system of the crayfish. 1. Spontaneous impulses. *J. Cell. Comp. Physiol.* **4**, 185–209.

Ramsay, J. A. (1953). Active transport of potassium by the Malpighian tubules of insects. *J. Exp. Biol.* **30**, 358–69.

Ripley, S. H. & Ewer, D. W. (1951). Peripheral inhibition in the locust. *Nature, Lond.*, **167**, 1066–7.

Roeder, K. D. (1948). The effect of potassium and calcium on the nervous system of the cockroach, *Periplaneta americana*. *J. cell. Comp. Physiol.* **31**, 327–38.

Stower, W. J. (1953). The colour types of the hoppers of *Schistocerca gregaria* (Forsk). London. Ph.D. thesis.

SLOW AND FAST RESPONSES IN COCKROACH LEG MUSCLE

By V. J. WILSON*

Department of Physiology, University of Illinois, Urbana, Illinois, U.S.A.†

(*Received* 28 *May* 1953)

INTRODUCTION

The multiple innervation of arthropod muscle has led to the suggestion that much of its electrical and mechanical activity is local in nature. It has also been shown by stimulation of isolated motor axons that several neuro-muscular systems may exist in the same crustacean muscle. Rapidly contracting fast systems, slow facilitating systems, and inhibitory mechanisms have been described (see Wiersma & Ripley, 1952). Wiersma & Wright (1947) maintain that all activity is non-propagated, but Katz & Kuffler (1946) have indicated that both local and propagated responses may be present. Katz (1949) considers that rapid twitches are brought about by stimulation of a fast, non-facilitating system, while graded activity depends on facilitating end-plate-like potentials, occurring in the vicinity of numerous nerve endings and associated with graded contractions. That propagation can take place within single fibres has been shown by Fatt & Katz (1951), who measured condition velocities in directly stimulated single muscle fibres with microelectrodes. While it is possible that different types of activity take place in the same muscle fibres (Wiersma & van Harreveld, 1938), the possibility remains that different muscle fibres are involved in the different types of contraction.

Neuro-muscular transmission in insects has been likened to the system described for Crustacea. Pringle (1939) described a non-facilitating fast system and a facilitating slow system in the extensor tibiae of *Periplaneta*, and believed that the difference between the responses was due to the number of muscle fibres involved in the contraction. Roeder & Weiant (1950), using external recording, have shown only an all-or-none fast system in the tergal muscle of the trochantin of *Periplaneta*, and believe that all electrical activity is local, with excitation being distributed over the muscle fibres by numerous nerve endings. Their reasoning is based on the positive sign of the externally recorded potentials (see also Wiersma & Wright, 1947), the presence of a multiple innervation (Roeder 1953), and the inexcitability of denervated muscle. However, the occurrence of propagation is suggested by the characteristics of the potentials recorded intracellularly from the flight muscles of flies by Boettiger & McCann (1953).

Since the muscles of insects as well as of Crustacea are innervated by a small number of motor neurones, the grading of contraction cannot depend on the involvement of large numbers of motor units. The different sizes of potentials

---

* National Science Foundation Fellow 1952–3.

† Present address: Department of Neurophysiology, Army Medical Service Graduate School, Washington 12, D.C., U.S.A.

recorded from the flexor tibiae of the roach by Hughes (1952) suggest that a small number of units are present in this muscle. Since the grading of activity may therefore depend largely on the interplay of slow and fast responses, it is of interest to study further the different responses first described by Pringle (1939). This is especially true in the light of recent work which has shown that slow and fast responses are present in other forms such as *Phascolosoma* (Prosser, Curtis & Travis, 1951), the frog (Kuffler & Williams, 1953), and the cat (Hunt & Kuffler, 1951). In the frog it has been shown with intracellular recording that the slow and fast responses take place in different muscle fibres (Kuffler & Williams, 1953). It seemed possible, therefore, that the responses of insect muscle could fit into a general scheme of motor activity, and the experiments to be described are an attempt to shed further light on neuro-muscular phenomena in *Periplaneta* by the use of intracellular recording techniques.

PROCEDURE

The flexor tibiae of *P. americana* was used in all experiments. The muscle was stimulated indirectly through the crural nerve in the coxa and the responses were recorded from exposed areas of the muscle from which the cuticle had been removed. The saline used throughout was the same as that used by Roeder (1948).

Both external and internal recording electrodes were used. In the former case recording was by means of fine platinum electrodes. In most instances recording was monophasic, the inert lead consisting of a platinum loop in contact with the saline in which the animal was bathed. The signal was amplified by a Grass P-4 condenser-coupled pre-amplifier and observed on a Dumont 208 oscilloscope. For internal recording the electrodes and circuits used were similar to those described by Nastuk & Hodgkin (1951). Fine electrodes with tips smaller than $1\,\mu$ were drawn from soft glass tubing and filled with 3 M-KCl. The signal was passed through a cathode follower stage and DC pre-amplifier, and photographed from the screen of a two-beam oscilloscope, consisting of two Dumont 304H oscilloscopes driving a common tube. In some instances microelectrodes too large for penetrations were used for recording of external activity.

RESULTS

(*a*) *External recording*

When the crural nerve was stimulated with single shocks of gradually increasing intensity the electrical response of the muscle was seen to grow in stepwise fashion. Three, and sometimes four, heights of potential were seen. In this type of response, shown in Fig. 1 A, it is clear that the increases in potential size are stepwise and not graded.

In some experiments the intensity of stimulation was kept constant while the frequency was increased. As a rule, little facilitation was seen in the frequency range used (up to eighty per second).

The externally recorded potentials are positive in many cases, but at other times negative potentials have been recorded (Fig. 1 B). The positive sign of the potentials was used by Roeder & Weiant (1950) as evidence for the absence of propagation. However, since both positive and negative potentials can be recorded, and since the shape of the potentials as well as their polarity varies with the placement of recording electrodes, it seems dangerous to attach too much significance to potential polarity.

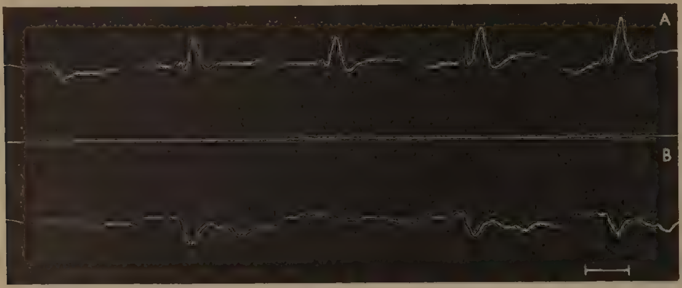

Fig. 1. External recording with large tip capillary inadequate for penetrations. A, gradual increase in intensity of stimulation of crural nerve. Four different steps in the height of the response can be seen. B, negative potentials obtained at low intensities of stimulation. Upward deflexion positive, time marker 10 msec.

(b) *Internal recording*

(1) *Criteria for penetration*

Three main criteria were employed in judging the validity of the experiments involving penetration of single fibres: (i) The first criterion was a sharp drop in the potential which was observed while the muscle mass was repeatedly stabbed with the electrode, and which was taken to indicate penetration of a fibre. (ii) The penetration was not counted as valid unless the potential, once recorded, remained steady while under observation. Although some drifting of the beam occasionally took place, on withdrawal of the electrode from the fibre the beam always returned to a point quite close to the zero level. (iii) Another criterion which was helpful in determining whether the electrode had indeed penetrated a fibre was the difference in the action potentials recorded externally and internally. The former were invariably small and complex, while the latter were large and simple, as will be described below.

(2) *The resting potential*

Records from a number of fibres showed that the resting potential varied from approximately 30 to 70 mV., with a mean of 45 ± 9 mV. (see Fig. 2A). It seems likely that this spread is due to some extent to the disparity between the ionic concentrations (especially potassium) of the saline used and the haemolymph. This will be discussed further, p. 288. It is interesting to point out that the saline used is a widely accepted one for the roach, although there is a tenfold difference between the saline and haemolymph potassium concentrations.

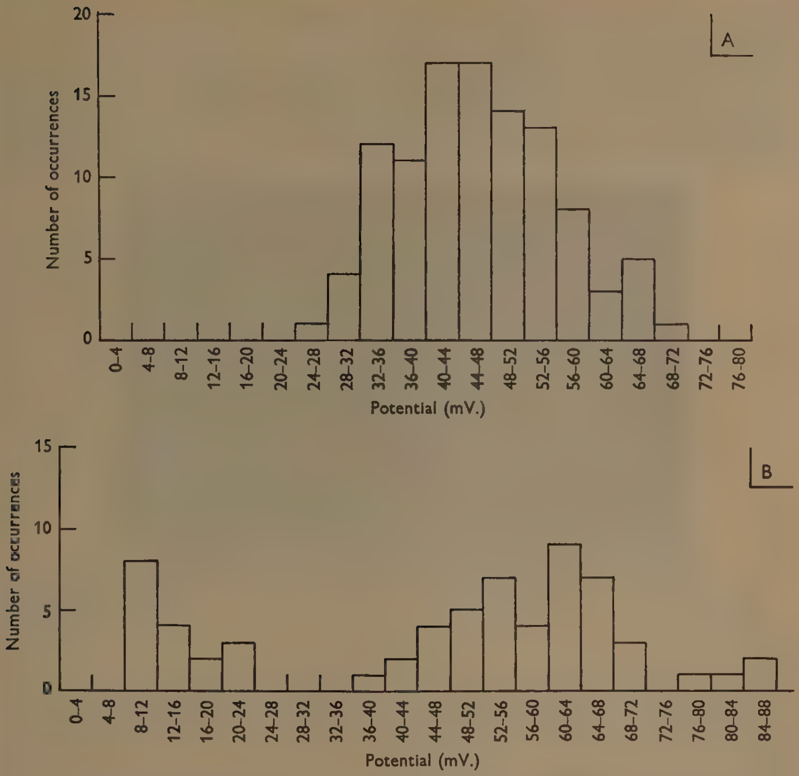

Fig. 2. Potentials obtained in microelectrode experiments. A, distribution of resting potentials. B, distribution of action potentials, fast-type potentials without overshoot omitted.

(3) *The action potential*

When the crural nerve was stimulated, different types of action potential were recorded in a series of penetrations. In forty-six cases large potentials were observed, varying in size from 40 to 85 mV. with 60% of the potentials between 55 and 70 mV., and showing an overshoot over the resting potential. These *type one* potentials, shown in Fig. 3A, had a duration of 3–4 msec., were invariably all-or-none, and showed no change when the frequency or intensity of the stimulus was changed within the same ranges which brought about changes in the externally recorded potentials.

Another group of action potentials was observed in seventeen cases, or approximately 18% of the penetrations. These *type two* potentials (Fig. 3B) were much smaller than the ones described above, being spread between 8 and 20 mV. Their time course varied, but was generally in the order of 6–8 msec., or twice the duration of the responses previously described. As will be shown later, these potentials are graded rather than all-or-none.

In addition to these two fairly well-defined groups of responses, twenty-nine potentials, varying in amplitude from 22 to 62 mV., were also observed. These potentials had an appearance similar to that of the potentials of larger size, except for the fact that the depolarization was not complete, and no overshoot was noted.

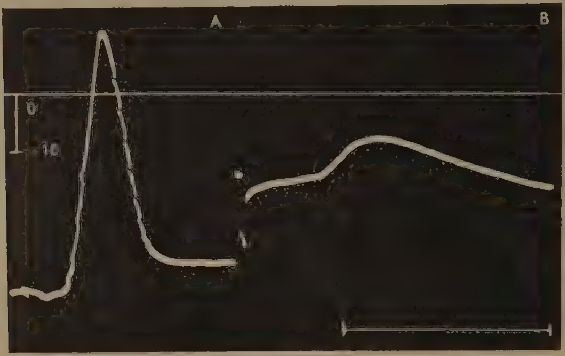

Fig. 3. A, example of *type one* potential, with overshoot. Resting potential 51 mV., action potential 70 mV., duration 4 msec. Electrode, 20 MΩ. B, example of *type two* potential (slight drift of beam). Resting potential 28 mV., action potential 11 mV., duration 9 msec. Voltage calibration, 10 mV., time marker 10 msec. Upward deflexion positive.

The time relations of these all-or-none potentials were the same as those of the *type one* potentials. The most reasonable explanation of these potentials of intermediate size is that they are actually the same response as the large potentials with overshoot, recorded under different conditions. It is likely that with some electrodes, although the fibre is penetrated, damage to the membrane occurs in the process of penetration, the potentials recorded therefore being smaller as a result of injury. Such potentials without overshoot have been observed in frog sartorius fast muscle fibres (Kuffler, 1953), and it has been previously pointed out that in nervous tissue the overshoot is a very labile phenomenon, disappearing rapidly on damage or penetration with a large electrode (Brooks & Fuortes, 1952). Another important similarity between these potentials and the *type one* response will be brought out in the experiments below.

The distribution of the *type one* and *type two* potentials is shown in Fig. 2B.

(*c*) *Experiments with paired shocks*

In an effort to clear up the relationships of the responses discussed above, a group of experiments were run in which paired shocks were applied to the nerve at different intervals.

(1) *Responses to paired shocks, recorded externally*

Fig. 4 shows the two potentials which are recorded from the flexor tibiae when the two stimuli are both just at threshold. As the two shocks are moved closer together, the second potential is seen to become reduced in size, disappearing when the two stimuli are about 2 msec. apart. This response is the same as that described in another muscle by Roeder & Weiant (1950).

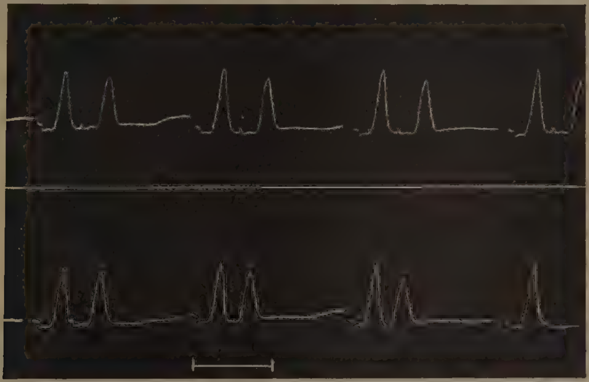

Fig. 4. Refractoriness following potential, recorded externally. In this experiment both stimuli were just of threshold intensity. The stimulus artifact preceding the first response is just visible at the beginning of the sweep, the second artifact can be seen super-imposed on the base-line and then on the first potential as the stimuli are brought closer together. The second potential disappears when the interval between the stimuli is 2 msec. Upward deflexion positive, time marker, 10 msec.

(2) *Responses to paired shocks, recorded internally*

In view of the complexity of the responses recorded externally from the muscle as a whole, it is much more profitable to study the behaviour of single fibres as they respond to two stimuli. Such experiments show that the *type one* potentials are definitely followed by an absolute and a relative refractory period. As the two stimuli are brought temporally closer and closer together the second response gradually decreases in size, no longer overshoots the resting potential, and disappears completely when the interval is decreased to about 2 msec. (Fig. 5). It is interesting to observe that the group of potentials which resemble the *type one* potentials in all respects except for the absence of an overshoot show exactly the same characteristics with regard to refractoriness. This is another of the reasons for the assumption that these two groups of potentials actually belong to the same type of response.

A sharp difference, however, is seen in the response of the *type two* potentials to the same kind of stimulation. While the *type one* responses are followed by a refractory period, and the second potential is therefore extinguished as it is brought closer to the first one, the *type two* potentials behave in a different manner (Fig. 6).

Here we see that as the two potentials move closer summation takes place. It is clear, therefore, that these potentials are not followed by a refractory period, but rather that facilitation takes place when stimuli are separated by very short intervals.

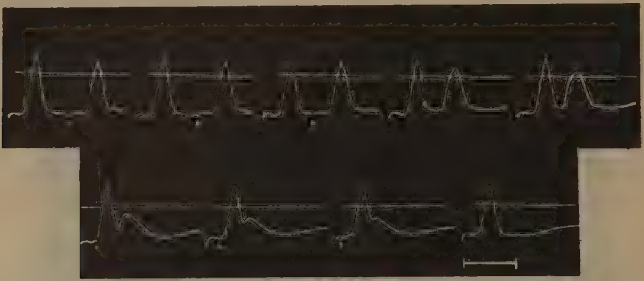

Fig. 5. Internal recording of double pulse experiment, showing refractory period of *type one* potential. First stimulus simultaneous with beginning of sweep. Note decrease in size of the second potential as the stimuli are moved closer together. Upward deflexion positive, time marker, 10 msec.

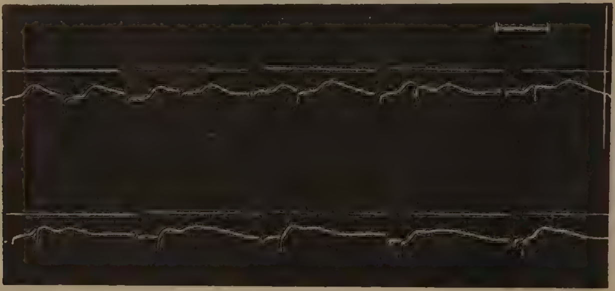

Fig. 6. Internally recorded double pulse experiment showing type two potentials. First stimulus artifact seen at the beginning of the sweep. Note lack of refractoriness as the potentials are moved progressively closer together. Upward deflexion positive, time marker, 10 msec.

DISCUSSION

Two divergent opinions exist concerning the responses of arthropod muscle. According to Wiersma & Wright (1947) and Roeder & Weiant (1950) all responses in crustacean and insect muscle are local in nature. Other authors contend that, while much of the activity is local, propagated electrical and mechanical events also occur (Katz & Kuffler, 1946; Fatt & Katz, 1951). The question of the locus of the diversified responses observed in crustacean muscle (Wiersma & Ripley, 1952) and to a lesser extent in insect muscle (Pringle, 1939) has not been conclusively settled. While there exists evidence that the difference between the slow and fast responses of crustacean and insect muscle is purely quantitative and involves the amount of contractile substance affected at one time within the same group of muscle fibres (van Harreveld, 1939; Pringle, 1939), the possibility remains that in insects as in the frog (Kuffler & Williams, 1953) the different responses are obtained by the excitation of specialized groups of muscle fibres.

There seems little doubt that the *type one* potentials described above are propagated. Such an assumption is based not on direct evidence, but on the all-or-none appearance of the potential recorded intracellularly, the presence of an overshoot over the resting potential, the short duration of the potential, and the presence of a refractory period following the response. The presence of an overshoot has invariably been associated with propagation in other tissues (Hodgkin, 1951). Furthermore, there has never been described a local response followed by a refractory period, or a propagated one without subsequent refractoriness.

Different lines of evidence have been used in an attempt to show that the potentials recorded from roach muscle are entirely local. First, Roeder & Weiant (1950) suggested that the positive polarity of the potentials recorded externally was indicative of local activity (see also Wiersma & Wright, 1947). As shown above, positive and negative potentials can be recorded from the flexor tibiae. In view of this fact, and of the fairly complex geometry of the system under study, it may be dangerous to attach decisive significance to potential polarity. Secondly, it has been shown that when the motor nerve is allowed to degenerate, insect muscle becomes unresponsive to stimulation, although its outward appearance remains unchanged (Roeder & Weiant, 1950). While this may indicate that excitation can be spread to all parts of each muscle fibre only by fine nerve branches, few conclusions can be drawn from this experiment because denervation is known to cause drastic changes in the excitability of many tissues (Cannon & Rosenblueth, 1949). Finally, just as has been done for Crustacea, it has been argued that the presence of a multiple innervation of muscle fibres in the muscles studied (Roeder & Weiant, 1950) makes propagation within single fibres unlikely, since excitation can easily be spread through the numerous fine branches of the motor nerve. According to Weiant (unpublished, see Roeder 1953) the nerve endings are separated by approximately 40μ. Should a similar innervation exist here, and should all activity consist of local responses around these endings similar to the end-plate potentials of vertebrate muscle, it might be expected that the size of the recorded potentials would vary with the locus of insertion of an electrode less than 1μ in diameter at the tip. An overshoot (representing the greatest possible response) would then be seen only on insertion right at a region of nerve ending, although it is hard to see why an overshoot, always associated with propagation, would be seen even then. The high frequency of occurrence of large potentials with an overshoot on random penetrations makes it even more unlikely that the potentials recorded are local.

On the whole it seems reasonable to assume that the *type one* potentials recorded in these experiments represent propagated activity in single fibres of the flexor tibiae of the roach, and that the same holds true for the similar potentials recorded from the flight muscle of flies by Boettiger & McCann (1953).

An entirely different interpretation presents itself in the case of the *type two* potentials previously described. Not only their small amplitude and somewhat longer duration, but also the fact that no refractoriness but rather some facilitation is seen when paired shocks are applied at close intervals, suggest that these potentials are local phenomena representing the activity of a slow system in the muscle studied. While it could be argued that these potentials are either due to damage of the fibre or abolition of the fast response through fatigue with only a local end-plate potential remaining, both of these possibilities are unlikely. The former is unlikely because it seems clear that on damage of the fast fibre with a large electrode the potential subsequently recorded is a fast-type potential without overshoot but followed by a refractory period similar to that of the undamaged fast response, and does not resemble the *type two* potential. The second possibility also seems improbable because the two types of potentials have been recorded from the same muscles while the preparation was in good condition and no evidence of fatigue had been seen. Finally, the difference between the two responses is emphasized by the fact that they have not been recorded in the same fibre, or at least with the same penetration, and that at no time has there been a shift from one type of response to the other. In general, it is likely not only that the *type one* and *type two* responses actually represent different phenomena taking place in the muscle, but also that these phenomena occur in different muscle fibres, some of which normally conduct while others do not.

Such a neuro-muscular system as described above is quite similar to the one described for the frog by Kuffler & Williams (1953). However, while those authors demonstrated that in the frog sartorius the fast, propagating fibres have a higher resting potential than the slow fibres giving only local responses, no such difference has been observed in the roach flexor tibiae.

An attempt can now be made to arrive at a picture of the grading of activity in the muscle studied. External recording has shown that three to four different heights of potential can be obtained from the flexor tibiae (see above, and Hughes, 1952). The changes from one height to another occur suddenly rather than gradually, and clearly represent the involvement of distinct motor units. The small number of motor units makes fine grading of activity according to the vertebrate plan impossible. Thus the fast system of this muscle is essentially divided into a small number of units which individually respond in an all-or-none manner, and are brought in as the intensity of stimulation of the nerve trunk is increased. The lack of facilitation in the fast system was pointed out by Pringle (1939), and is again seen here with both external and internal recording. Grading of activity in the fast system seems to depend entirely on the separate or joint activation of a small number of motor units, and on mechanical summation at raised frequencies of stimulation.

Finer grading of movement is evidently made possible by the presence of a facilitating slow system which, as in the frog, may be mainly involved in 'tonic' movements.

That the resting potentials show such a wide range of distribution is probably due largely to the disparity between the potassium concentrations of the haemolymph and of the saline used in these experiments (27 mM./l. as against 2·7 mM/l.).

This would lead to uneven mixing between these two media, with the result that some muscle fibres would be exposed to higher external potassium concentration than others. It has been shown recently that in *Locusta* muscle resting potentials depend to some extent on the external potassium concentration (Hoyle, 1953), and the values obtained in the experiments described above fall within the range expected from the graph presented by Hoyle.

On the whole, therefore, it seems that the neuro-muscular system of the roach is similar to that described in other forms. The slow and fast systems in *Periplaneta* seem to be restricted to specialized muscle fibres, and the same is now found to be true in the frog (Kuffler & Williams, 1953), although it had been previously thought that in both these animals the same fibres were involved in the two responses (Pringle, 1939; Kuffler & Gerard, 1947). This suggests that a re-examination of crustacean neuro-muscular preparations with intracellular electrodes might be of great value, especially in the claw closer preparation described in the crayfish by van Harreveld & Wiersma (1936), where the easy separation of the slow and fast motor axons makes conditions ideal for a study of the two neuro-muscular systems. Evidence from comparative studies on several forms, as well as the work of Fatt & Katz (1951) and Katz & Kuffler (1946), suggests that the responses of Crustacea may be quite similar to those observed in insects and Amphibia. The main difference noted until now between the fast system of insects and that of Crustacea is that while the potentials in the former are followed by a refractory period (above, and Roeder & Weiant, 1950) those in Crustacea are not (van Harreveld & Wiersma, 1936). This may indicate an important difference between neuro-muscular phenomena in these two classes, and in any case shows that generalizations from higher Crustacea to insects are, on present evidence, quite unjustified.

SUMMARY

1. Intracellular recording from individual muscle fibres of the flexor tibiae of the cockroach shows two types of action potentials. One of these represents fast fibre activity, while the other represents slow fibre activity in this muscle.

2. The fast response consists of an all-or-none potential varying from 40 to 85 mV., with 60% of the responses between 55 and 70 mV. This potential has an overshoot over the resting potential, and is followed by an absolute and a relative refractory period. For these reasons it is considered likely that the potential is a propagated one. The slow response is a small, facilitating potential, varying from 8 to 20 mV., and is quite likely a local phenomenon.

3. All the evidence indicates that the two types of activity do not take place in the same muscle fibres, but rather represent the responses of two different types of fibres, as is the case in some amphibian muscles.

4. Gradation of activity in the fast system seems to depend solely on the activation of a very small number of motor units and on mechanical summation. The response of this system shows little, if any, facilitation as the frequency of stimulation is increased. The facilitating slow system may be concerned with slower, more finely graded movements.

5. The resting potential in roach muscle has an amplitude of 45 ± 9 mV., which falls within the range expected on the basis of the intracellular and extracellular potassium concentrations.

6. Slow and fast fibre activity in insects and Crustacea is discussed, and a revision of the earlier work on Crustacea with the use of intracellular recording is suggested.

I should like to thank Dr C. L. Prosser, under whose guidance this work was carried out, for his many suggestions and continued interest in the problem. I am also very grateful to Dr Kenneth D. Roeder, Tufts College, whose guidance and enthusiasm are responsible for my interest in this field.

REFERENCES

BOETTIGER, E. G. & MCCANN, F. (1953). Single fibre action potentials in insect fibrillar muscle. *Fed. Proc.* **12**, 17.

BROOKS, C. McC. & FUORTES, M. G. F. (1952). Excitation, conduction and synaptic transmission in the nervous system. *Ann. Rev. Physiol.* **14**, 363–90.

CANNON, W. B. & ROSENBLUETH, A. (1949). *The Supersensitivity of Denervated Structures.* New York: The Macmillan Company.

FATT, P. & KATZ, B. (1951). Conduction of impulses in crustacean muscle fibres. *J. Physiol.* **115**, 320–70.

HARREVELD, A. VAN (1939). The nerve supply of doubly and triply innervated muscles related to their function. *J. Comp. Neurol.* **70**, 267–84.

HARREVELD, A. VAN & WIERSMA, C. A. G. (1936). The double motor innervation of the adductor muscle in the claw of the crayfish. *J. Physiol.* **88**, 78–99.

HODGKIN, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339–409.

HOYLE, G. (1953). Potassium ions and insect nerve muscle. *J. Exp. Biol.* **30**, 121–35.

HUGHES, G. M. (1952). Differential effects of direct current on insect ganglia. *J. Exp. Biol.* **29**, 387–402.

HUNT, C. C. & KUFFLER, S. W. (1951). Further study of efferent small-nerve fibres to mammalian muscle spindles. Multiple spindle innervation and activity during contraction. *J. Physiol.* **113**, 283–97.

KATZ, B. (1949). Neuro-muscular transmission in invertebrates. *Biol. Rev.* **24**, 1–20.

KATZ, B. & KUFFLER, S. W. (1946). Excitation of the nerve muscle system in Crustacea. *Proc. Roy. Soc.* B, **133**, 374–89.

KUFFLER, S. W. Personal communication.

KUFFLER, S. W. & GERARD, R. W. (1947). The small-nerve motor system to skeletal muscle. *J. Neurophysiol.* **10**, 383–94.

KUFFLER, S. W. & VAUGHAN WILLIAMS, E. M. (1953). Small-nerve innervation pattern of frog's skeletal muscle. Membrane characteristics of small-nerve junction potentials (S.J.P.). *J. Physiol.* (in the Press).

NASTUK, W. L. & HODGKIN, A. L. (1951). The electrical activity of single muscle fibres. *J. Cell. Comp. Physiol.* **35**, 39–73.

PRINGLE, J. W. S. (1939). The motor mechanism of the insect leg. *J. Exp. Biol.* **16**, 220–31.

PROSSER, C. L., CURTIS, H. J. & TRAVIS, D. M. (1951). Action potentials from some invertebrate non-striated muscles. *J. Cell. Comp. Physiol.* **38**, 299–319.

ROEDER, K. D. (1948). The effects of potassium and calcium on the nervous system of the cockroach, *Periplaneta Americana. J. Cell. Comp. Physiol.* **31**, 327–38.

ROEDER, K. D. (ed.) (1953). *Insect Physiology.* New York: John Wiley and Sons, Inc.

ROEDER, K. D. & WEIANT, E. A. (1950). The electrical and mechanical events of neuro-muscular transmission in the cockroach, *Periplaneta Americana L. J. Exp. Biol.* **27**, 1–13.

WIERSMA, C. A. G. & HARREVELD, A. VAN (1938). The influence of the frequency of stimulation on the slow and fast contraction in crustacean muscle. *Physiol. Zool.* **11**, 75–81.

WIERSMA, C. A. G. & RIPLEY, S. H. (1952). Innervation patterns of crustacean limbs. *Physiol. Comp. Oecol.* **2**, 391–405.

WIERSMA, C. A. G. & WRIGHT, E. B. (1947). The nature of the action potentials of crustacean muscles. *J. Exp. Biol.* **23**, 205–12.

OBSERVATIONS ON THE MECHANISM OF THE OPENING OF THE VALVES OF A BURROWING LAMELLIBRANCH, *MYA ARENARIA*

By E. R. TRUEMAN
Department of Zoology, University College, Hull

(*Received* 29 *October* 1953)

INTRODUCTION

In most lamellibranchs the shell consists of a pair of valves joined dorsally by a horny structure, the ligament, which, when the valves are closed, is under strain. The force so derived tends to open the valves and has been termed the opening moment of the ligament (Trueman, 1953). Measurement of the opening moment of a range of lamellibranchs in terms of the size of the individual has shown that there appears to be little relation between the strength of the ligament and the habitat. In certain attached and free-swimming lamellibranchs, e.g. *Ostrea* or *Pecten*, the ligament is apparently the sole mechanism used to open the valves. In *Mya arenaria*, a lamellibranch which burrows deeply, the opening moment and the conditions of the opening of the valves by the ligament have been investigated and the results recorded in this paper. The opening moment of this ligament is inadequate to open the valves when the animal is buried. Accordingly, a preliminary investigation has been made of a variety of marine soils with reference to the opening of the valves of *M. arenaria*. These results, together with those of an investigation of the means by which this species opens its valves against known resistances, are discussed in this paper. For these investigations *M. arenaria* has been used chiefly because it is readily available, is a deep burrower and is fairly large.

THE OPERATION OF THE LIGAMENT

In *M. arenaria* the outer and inner layers of the ligament occupy a restricted position between the right valve and the chondrophore of the left valve. The peculiar form and location of the ligament allow it to function in a dual role. It causes the valves to open about the pivotal axis of the ligament in the normal manner, and it also allows the valves to rock about a dorso-ventral axis (Fig. 1, *DV*) so that the shell margins may be drawn together or divaricated at either end. It is hoped to publish shortly an account of the functional morphology of this ligament.

By removal of the tissues and the application of known weights to the centre of the surface of one valve, a method recently described in detail (Trueman, 1953), the closing moment of the ligament of *M. arenaria* has been determined and the opening moment deduced. The closing moment may be defined as the force applied to the valves which just closes them. To obtain some basis of comparison for the closing moment of the ligament of different species, it was found convenient to relate the moment to the shell volume (Trueman, 1953). The ratios determined for ten specimens of *M. arenaria* were, closing moment 191 g.mm./ml. (S.E. ± 11·3), opening moment 174 g.mm./ml. These figures indicate that this ligament is not very powerful and that it has a weaker opening moment than that of certain attached species, e.g. *Mytilus edulis* and *Ostrea edulis*, opening moment 660 and 370 g.mm./ml. respectively (Trueman, 1953, Table 1).

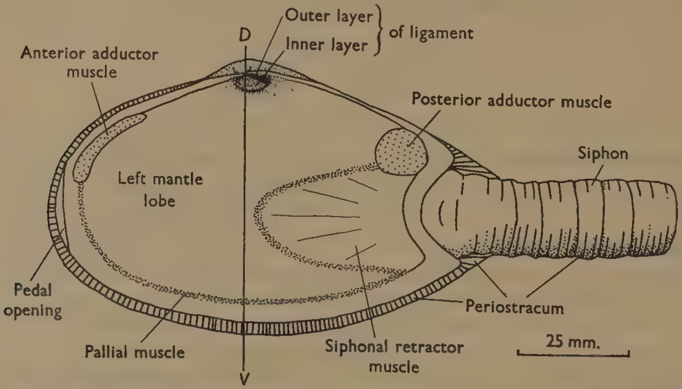

Fig. 1. *Mya arenaria* with the left valve removed, showing the left mantle lobe and the ligament sunk beneath the umbo of the right valve. The line *DV* indicates the dorso-ventral axis about which the rocking movement of the valves takes place.

In a form that burrows, such as *Mya*, there is probably a relationship between the surface area of the valves and the resistance of the soil to the opening of the shell. Accordingly, it is convenient to express the opening and closing moments in terms of the projected surface area of the valves. The ratios so obtained were closing moment 3·8 g.mm./mm.^{2} (mean of ten specimens, S.E. ± 0·25) and opening moment 3·4 g.mm./mm.^{2}

If a load-extension curve is plotted for the loading and unloading of an elastic material the form of the curve is a hysteresis loop. A similar curve may be drawn for the ligament, the load being represented by the moment applied to the valves and the extension by the angle of gape (Trueman, 1953). When considering *M. arenaria* it is convenient to express the load in terms of the projected surface area of the valves in the same manner as the closing and opening moments. The loading curve for this species (Fig. 2, *AB*) is a nearly linear relationship similar to that recorded for certain other lamellibranchs (Trueman, 1953). The unloading curve (Fig. 2, *BCD*) shows little change of gape when the applied moment is slightly reduced. The valves commence to gape (Fig. 2, *C*) only when the thrust of the ligament exceeds the pressure on the valves, thus point *C* on the curve represents the opening moment of the ligament. The remainder of the unloading curve (Fig. 2, *DC*) is similar to the loading curve.

The difference in the opening and closing moments is some measure of the internal resistance of the structure. The smaller the area (*ABCD*) of such a loop the lower is the work-loss of the ligament and the higher its efficiency. The ligaments of *Pecten* or *Chlamys* have notably small loops (Trueman, 1953), whereas that of *Mya arenaria* is comparable with those of many other lamellibranchs. This may be illustrated numerically by the estimation of the percentage damping of the ligament in a complete loading and unloading cycle according to the method of Davies (1952). In *M. arenaria* the percentage damping is 21 %, a value similar to that for the ligament of *Lutraria lutraria* or *Mytilus edulis*, while the figure for the ligament of *Chlamys opercularis* is much lower (4·5 %).

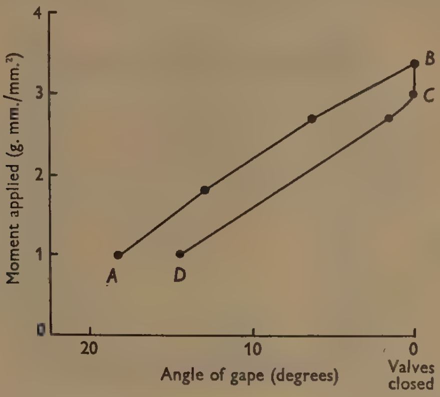

Fig. 2. Graph showing the relation of the moment applied to the valve (expressed as a ratio between the moment about the pivotal axis and the projected surface area of a valve) and the angle of gape of the valves of *Mya arenaria*. *AB* represents the loading curve and *BCD* the unloading curve.

In life the valves of *Mya* rarely become completely closed and probably never attain the maximum gape observed in these experiments with the tissues removed from the shell. The opening and closing of the valves would thus occur on the central region of the curve (Fig. 2) as a complete unloading-loading cycle. Further discussion of the observations made on the operation of the ligament is deferred until after a consideration of certain other factors.

THE EFFECT OF THE SUBSTRATUM ON THE OPENING OF THE VALVES

Many lamellibranchs burrow in sandy or muddy soils in which their valves open and close to varying extents against some resistance of the soil. Although the properties of marine and estuarine soils have recently been studied (Boswell, 1948), particularly with reference to the burrowing of *Arenicola* (Chapman, 1949; Chapman & Newell, 1947), there is little relevant information concerning the burrowing of lamellibranchs. Accordingly, experiments have been carried out to ascertain the resistance of various soils to the opening of the valves of *Mya*. Determinations were made of the resistance of the soil using lamellibranch shells rather than equipment such as a dilatancy viscometer since in this way one can approach the problem under investigation more closely. It was convenient to use the valves of *M. arenaria*, both on account of their fairly large size and the possibility of removing the outer and inner layers of the ligament without damaging the fusion layer, which then holds the valves together. Further experiments were carried out using the valves of *Cardium edule* and *Cyprina islandica* with comparable results.

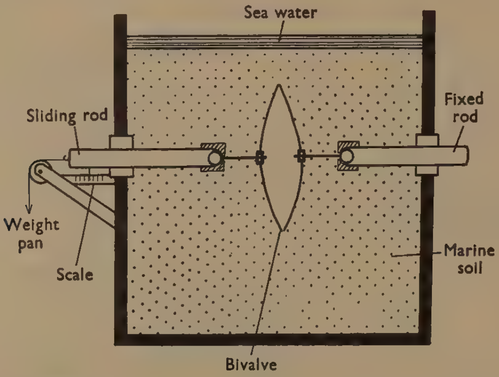

Fig. 3. Diagrammatic section of the apparatus used to determine the resistance of various soils to the opening of the valves of *Mya*.

For this investigation the equipment shown in Fig. 3 was constructed. It consisted of a cubical wooden box, of side 1 ft., filled with sand or mud. In this a specimen of *Mya* was placed, tissues and all the ligament, apart from the fusion layer, removed. A small hole was drilled in the centre of each valve. Protruding into the box were two rods, one sliding and the other fixed, with ball and socket joints connecting the rods to thin bolts attached to the specimens by holes in the valves. The application of weights to the sliding rod tended to cause the valves to gape. Any movement of the valve to which the sliding rod was attached, the other valve being held firm, was measured on a scale outside the box. Thus the angle of gape of the valves and the force required to produce it could be determined. It was found necessary to put a thin rubber sheath over the sliding rod to exclude sand particles. The procedure was to place the prepared specimen of *Mya* in the box and to cover the specimen with the soil under investigation. The soil was covered with sea water so that it was always saturated with water. Before weights were added to the sliding rod a standard procedure of tapping the box fairly vigorously 40 times and allowing it to stand for about 10 min. was carried out. In the results given here the readings of the valve movement were always taken immediately after the application of the weight and the successive additions of weight were made within 2 min. of each other. The results for sand, mud and various mixtures have been plotted graphically. The weight applied is expressed as a moment about the pivotal axis per mm.^{2} of the projected surface area of the valve in the same manner as the opening moment has been determined. The movement of the sliding rod is converted to degrees of gape of the valves.

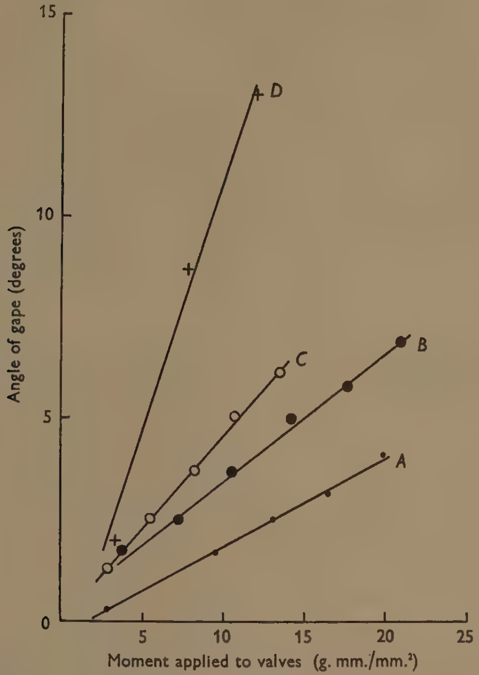

Fig. 4. Graph showing the relation between applied moment and angle gape of the valves of *M. arenaria* in various soils. *A*, •—•—•, clean sand from Kames Bay, Millport, valves covered by 1 in. of sand; *B*, ●—●—●, muddy sand from Balloch Bay, Isle of Cumbrae, valves covered by 1½ in. of muddy sand; *C*, ○—○—○, mixture of clean sand (Kames Bay) and mud (10% by volume), valves covered by ½ in. of mixture; D, +—+—+, offshore mud, valves covered by ½ in. of mud.

Fig. 4 shows a series of typical curves for the opening of the valves of *M. arenaria* in various soils, obtained as described above. In drawing these curves allowance has been made for the resistance of the sliding rod to movement, ascertained in the various media by the addition of weights to the rod without a lamellibranch attached. The fine sand sample used in these experiments was obtained from the centre of Kames Bay, Millport, near to the low-water mark. This sand consists mainly (about 90%) of particles of between 0·5 and 0·2 mm. with small silt and clay fractions (Watkins, 1942). The muddy sand was obtained from near low water in Balloch Bay, Isle of Cumbrae and the mud sample was taken from a point between the Old

Pier, Millport, and the Small Cumbrae. Mixtures of the Kames Bay sand and this mud were also used.

The curves shown in Fig. 4 all give a linear relationship between the applied moment and the angle of gape of the valves, and indicate that the sand used was more resistant to the opening of the valves than the mud; muddy sand and mud-sand mixtures were intermediate. There is no sign of any break in these curves such as Freundlich & Roder (1938) describe as characteristic of a dilatant material. These authors observe that when plotting the speed of the sphere of a dilatancy viscometer against the applied weight, a thixotropic substance gives a straight-line graph, cutting the abscissa to the right of the origin. Movement only commences after a certain minimum force is applied, the speed then being proportionate to weight as for a Newtonian liquid. The curves shown in Fig. 4 pass close to the point of origin, apart from that for the sample of sand from Kames Bay which cuts the abscissa to the right of the point of origin. Thus the soils examined behave either as Newtonian liquids or as if thixotropic. Boswell (1948) has discussed the properties of certain shore and estuarine sediments, and considers that muddy sediments within the intertidal belt are in the thixotropic state.

The valves of a living *M. arenaria* frequently gape to an angle of about 10 degrees. The steady moment that has to be applied to open the valves in sand to this degree of gape (approximately 45 g.mm./mm.^{2}) is far in excess of the opening moment of the ligament. In the sandy mud of Balloch Bay this moment is reduced to 30 g.mm./mm.^{2} and in the offshore mud sample to 9 g.mm./mm.^{2} Estuarine or intertidal mud is very probably more resistant than the latter. Boswell (1948) considers shore muds as being less fluid than those of estuaries or offshore areas, probably because they are partially dried out between tides.

The depth of burial has some effect on the opening moment required to open the valves. A specimen of *M. arenaria* buried with the valves covered by 1 in. of sand required a moment of 11 g.mm./mm.^{2} applied to produce a gape of 2 degrees. The same specimen covered by 4 in. of sand required an applied moment of 16 g.mm./mm.^{2} for a similar gape.

An important property of thixotropic substances is that on stirring or agitation they tend to liquefy, undergoing a gel-sol transformation. In dilatant substances, on the other hand, the resistance to shearing stress is considerable, and agitation generally has the effect of solidifying a sol. Agitation of thixotropic sand causes its liquefaction and when agitation ceases it re-sets quite rapidly, in less than a minute according to Chapman & Newell (1947). These authors have shown how *Arenicola* utilizes the thixotropic properties of muddy sand in burrowing, reducing the resistance by agitation with the proboscis. The possibility of lamellibranchs reducing the resistance to the opening of the valves by temporary liquefaction of the soil should not be overlooked. Agitation by means of the sliding rod (Fig. 3) proved unsatisfactory, the valves becoming fractured. The tapping of the box produced sufficient agitation of the soil to allow the valves to open much more readily. Fig. 5 shows a normal curve for the application of a steady force to the valves of *M. arenaria* in a mixture of sand and mud (10% mud by volume). Moderately hard tapping of the box at point *A* gives a much greater gape for the same applied moment. Liquefaction of the soil around the shell allows the valves to open more readily than when the soil is in a less fluid condition. The means by which this may be carried out is discussed later.

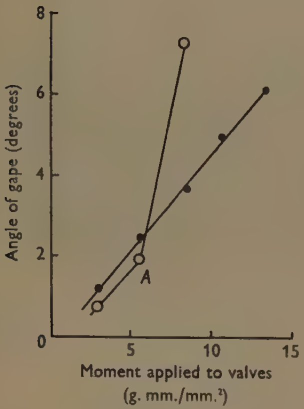

Fig. 5.

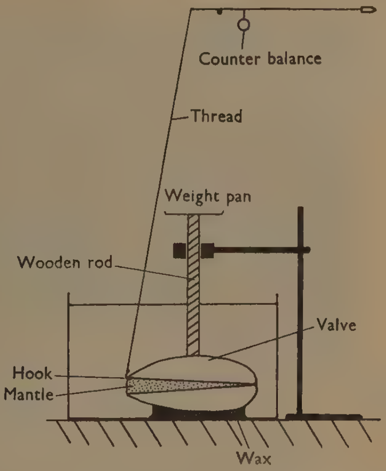

Fig. 6.

Fig. 5. Graph showing the reduction of the resistance of a mixture of sand and mud, as used in Fig. 4*C*, to the opening of the valves after agitation of the box by tapping at point *A*. ●—●—●, normal curve; ○—○—○, similar curve with agitation.

Fig. 6. Diagram of the apparatus used to record the movement of the valves of *M. arenaria* with various loads applied to the upper valve.

THE EXPERIMENTAL LOADING OF THE VALVES OF *MYA ARENARIA*

The determination of the resistance of various soils to the opening of the valves of lamellibranchs, as described above, has shown that the opening moment of the ligament of *M. arenaria* is quite inadequate to open the valves if applied as a steady force. Experiments have therefore been carried out to ascertain against what resistance this species is able to open its valves and observations have been made on the methods by which the opening moment of the ligament may be supplemented.

In these experiments the apparatus shown in Fig. 6. was used. Specimens of *Mya* were placed in a tank of sea water with a valve attached to the bottom by means of black wax, such as is commonly used in dissecting dishes. Weights could then be applied to the centre of the surface of the upper valve by means of a wooden rod extending to a weight pan above the surface of the water. The movements of the upper valve were recorded by the insertion of a small hook at the ventral margin of the valve connected by a fine thread to a gimbal lever, counterbalanced slightly so that there was always slight tension on the thread.

The records of the activity of specimens of *M. arenaria* were taken from twelve individuals, each one being generally kept in the apparatus for periods of 2 or 3 days, although some maintained activity for over a week.

The normal activity pattern of *Mya* was recorded either without any load on the valves or with a small load, such as the weight of the wooden rod (approximately 100 g.). This activity pattern is shown in Fig. 7. It may be noted that the valves were frequently closed rapidly, indicated by the downward sweep of the trace, from a position of gape. After rapid closure the opening of the valves is rather more gradual. This closing and opening of the valves is somewhat similar to that recorded by Nelson (1921) for *Ostrea virginica,* who related the closing of the shell to the turbidity of the water. The rapid closure of the valves of *Mya arenaria* is probably associated with the ejection of waste matter from the mantle cavity. The first part of the trace shown in Fig. 7 represents the movements of the valves of *Mya* with a load of 100 g. applied as described above, and at point *A* the load was increased to 300 g. Following such an increase the mean angle of gape of the valves is slightly reduced and the closing of the valves becomes less frequent.

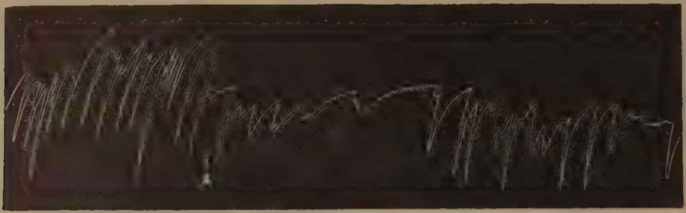

Fig. 7. Extract of the record of the movement of the valves of *M. arenaria* showing the closing pattern of activity. At point *A* the load applied to the valves was increased from 100 to 300 g. The closed position of the valves in this and subsequent recordings is at the bottom of the trace. Duration of extract 4 hr. Read all extracts from left to right.

The maximum gape of the valves with a load of 300 g. applied, as recorded in Fig. 7, is 6·5 degrees. At this gape the opening moment of the ligament of a typical specimen of *M. arenaria* may be estimated, using the curve in Fig. 2, to be 2 g.mm./mm.^{2} In the specimen being used this is equivalent to an upward thrust of about 300 g. at the point of application of the load. This corresponds with the applied load of 300 g. Similar results have been obtained with other specimens under identical conditions. It may be suggested that this activity, which may be termed a closing pattern, occurs when the load on the valves or the resistance to their opening does not exceed the opening moment of the ligament. The valves may thus open readily to an optimum angle of gape by means of the elasticity of the ligament.

If the load on the valves is increased to a value exceeding the opening moment of the ligament a different activity pattern is obtained, which may be termed the opening pattern (Fig. 8). In place of the repeated closure of the valves from a gaping position, the valves are rapidly opened and then closed, in a period of from 10 to 20 sec., from a closed position of rest. This activity occurs with approximately the same frequency as the closing pattern, but it may be clearly observed from the trace shown in Fig. 8 that the opening of the valves occurs in a fairly regular pattern of activity with two or three openings in rapid succession, followed by a short period of inactivity.

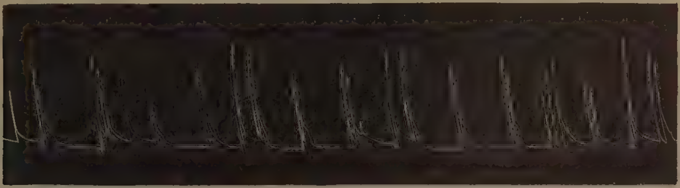

Fig. 8. Extract of a record of the movement of the valves of *M. arenaria* showing the opening pattern of activity with an applied load of 600 g. Duration of extract 2½ hr.

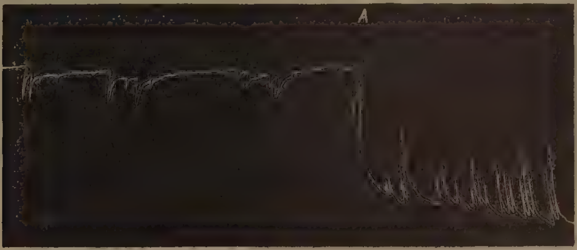

Fig. 9. Extract of a record of the movement of the valves of *M. arenaria* showing a change from the closing to the opening pattern at point *A*, where the load applied to the valves was increased from 300 to 600 g. Duration of extract 1¾ hr.

The opening pattern of activity was frequently observed with loads of up to 2000 g. (equivalent to approximately 20 g.mm./mm.^{2}) applied to the valves. Such a load is about 6 times the available thrust of the ligament. It should be noted that the rapid opening of the valves is not sustained, so that any force applied to open the valves is of a temporary nature.

The rapid change from the closing to the opening pattern is shown in Fig. 9. Up to point *A* the valves were under a load of 300 g., their angle of gape was 8 degrees and a closing pattern of small amplitude may be observed. At point *A*, when the load was increased to 600 g., the valves closed to a gape of about 1 degree and the opening pattern appeared.

The origin of the force additional to the opening moment of the ligament, which contributes to the opening of the valves, was next investigated. Yonge (1949) observes that in very deep burrowers, such as *Mya* and *Lutraria*, the ventral mantle margins are greatly enlarged and probably, when filled with blood, assist the ligament in pushing the valves apart against the substratum. Accordingly, an attempt was made to introduce a cannula or needle into the ventral mantle margin to endeavour to record changes in blood pressure in relation to the opening of the valves against loads. No satisfactory results were obtained.

It was considered possible that a force could be exerted on the valves by means of water in the mantle cavity. A small glass cannula, attached to a water manometer, was introduced into the mantle cavity through the inhalent siphon. Changes of water pressure were then observed when the shell opened under loads in excess of the strength of the ligament. Similar results were generally obtained by placing the cannula in the exhalent siphon. The activity of the specimens used for this investigation was more nearly normal when the cannula was introduced into the mantle cavity through the pedal opening. The records given in this paper were made by the use of this method. The cannula was connected directly to a narrow glass tube with a small float suspended at the surface of the water and attached to a frontal writing lever to record the changes in water pressure. In this way simultaneous recordings of valve movement and change of pressure in the mantle cavity were made (Figs. 10, 11).

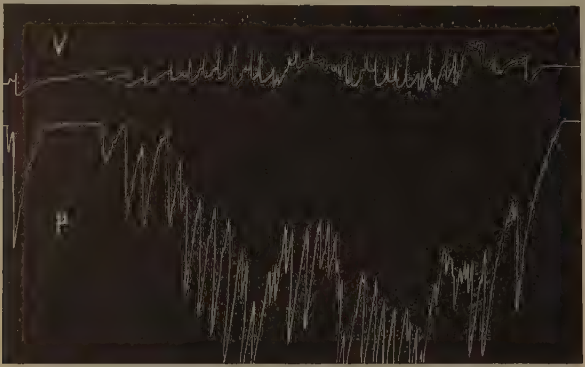

Fig. 10. Extract of a simultaneous recording of the movement of the valves (*V*) and of the changes in water pressure in the mantle cavity (*P*) of *M. arenaria*. The downward movement of the pressure trace indicates increase in pressure. Applied load 500 g. Duration of extract 3½ hr.

Fig. 10 shows the movements of the upper valve (*V*), when loaded with 500 g. as described above, and the corresponding fluctuations of pressure (*P*) in the mantle cavity of a small specimen of *Mya arenaria* (70 mm. overall length). The valve movement (*V*) shows the opening pattern, and corresponding with each peak there is a downward movement of the pressure trace (*P*), indicating an increase in pressure of the water in the mantle cavity. The relation is more clearly shown on the faster trace (Fig. 11) where it may be noted that whereas the increase in pressure is rapid its return is much slower. This was possibly due to the use of a relatively wide tube (10 mm. diameter), without any outlet, to record the fluctuations of pressure. The absence of any outlet maintained the mantle cavity as a closed system apart from the siphons.

More accurate direct observations on the pressure changes were made by the use of a simple manometer of the kind described by Chapman & Newell (1947) for measuring the pressure of the body fluid of *Arenicola*. The manometer tube used was of 1 mm. diameter, so that the volume change, when the pressure varied, was small in relation to the total volume of water in the mantle cavity. Observations using this manometer in conjunction with recordings of the valve movement showed increase of pressure occurring just prior to the opening of the valves. The increase of pressure took approximately 10 sec., the decrease 15 sec. and the maximum pressure was maintained for only 2 or 3 sec. The valves began to close immediately following the commencement of the decrease in pressure. The increase in water pressure in the mantle cavity may thus be directly related to the opening of the valves for short periods against loads in excess of the opening moment of the ligament.

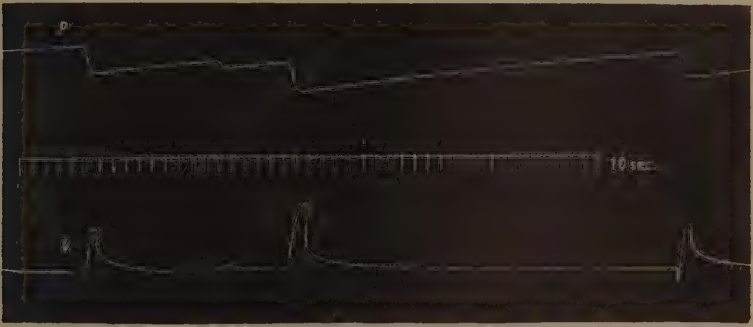

Fig. 11. Extract of a simultaneous recording of the movement of the valves (*V*) and of the changes in water pressure (*P*) in the mantle cavity of *M. arenaria*. Applied load 1100 g.

One typical example illustrates the relationship between the load, the opening moment of the ligament and the hydrostatic pressure. A single peak indicating the rapid opening and closing of the valves of a small specimen of *Mya* (62 mm. overall length) under a load of 1100 g. applied 25 mm. from the hinge axis, was observed on the kymograph and a simultaneous increase of water pressure in the mantle cavity of 30 cm. of sea-water noted. The opening moment of the ligament in such circumstances would not exceed 3 g. mm./mm.^{2}. Taking into account the projected surface area of the valve of 2100 mm.^{2}, this represents an opening moment about the hinge axis of 6300 g. mm. The load applied represents a loading moment of 27,500 g. mm. There must therefore be an opening moment applied to the valves equal to the difference between the latter figures, a moment of 21,200 g. mm. The observed increase in pressure in the mantle cavity represents a force of 0·3 g./mm.^{2}, applied to the part of the valves corresponding to the extent of the mantle cavity. This is just less than two-thirds of the projected surface area of each valve. Thus the force applied to the valves by the pressure in the mantle cavity is approximately

800 g. at a mean distance from the hinge axis of 25 mm. Therefore the opening moment derived from the mantle cavity is about 20,000 g. mm., which, with the addition of the opening moment of the ligament, is approximately equal to the load applied. The hydrostatic pressure is obviously of the correct order of magnitude to cause the opening of the valves in the conditions described.

Using the specimen considered above the maximum load against which the valves could open was found to be 2200 g., equivalent to a loading moment of 55,000 g. mm., or expressed in terms of projected surface area of the valves a maximum of 26 g. mm./mm.^{2} This appears to be approximately the maximum loading moment against which specimens of *M. arenaria* can open their valves.

Large fluctuations of pressure were not observed when the valves were not loaded in excess of the opening moment of the ligament, but small increases of short duration, of the order of 3 or 4 cm. of sea water were recorded both in unloaded and loaded specimens. With such pressure fluctuations there was frequently no movement of the valves recorded. Sudden increase in pressure in the mantle cavity may be related to the ejection of accumulated waste material from the inhalent region of the mantle cavity through the inhalent siphon, which is commonly extended a long way above the shell.

The water pressure recorded in the mantle cavity may also be related to siphonal movement. A sustained increase of pressure, 3 cm. sea water for more than 5 min., was observed to be related to the extension of the siphon, but further observations are required on this aspect. Tactile stimulation of the siphon causes its contraction. The anterior ends of the valves are drawn together and the posterior region divaricates to allow the withdrawal of the siphon. This generally gives rise to a temporary increase in pressure (around 15 cm.) in the mantle cavity. It may be observed that such a movement of the valves about a dorso-ventral axis through the ligament (Fig. 1) is a phenomenon which is associated with the localized arrangement of the ligament in *Mya*. Rocking about this axis may be of importance in the mechanism by which the increase of the hydrostatic pressure in the mantle cavity may be produced. Any such mechanism would require the temporary closing of all mantle openings and powerful muscular contraction, possibly by the retraction of the siphons slightly, both to raise the pressure of water in the mantle cavity and to compensate for any increase in volume of the cavity when the valves open. The ventral mantle margin bulges quite prominently, tactile stimulation causing its withdrawal and a small temporary increase in pressure in the mantle cavity. Sections of the mantle edge show the fusion of the inner mantle lobes and the powerful pallial muscles attached between the margins of the valves. These muscles may be related to the change in the hydrostatic pressure in the mantle cavity. But further work is required to establish the exact means whereby the hydrostatic pressure is developed.

DISCUSSION

In this paper an attempt has been made to investigate certain of the mechanical factors involved in the opening and closing of the valves of a burrowing lamellibranch, *Mya arenaria*, in various marine soils.

The ligament causes the valves to open when the adductor muscles relax. The opening moment of the ligament of *M. arenaria* and that of many other lamellibranchs that burrrow, e.g. *Tellina tenuis*, *Lutraria lutraria*, is no more powerful, and often less so, than that of attached or free-swimming species, e.g. *Mytilus edulis*, *Pecten maximus*. Since the former have to overcome some resistance of the soil when opening their valves, an investigation has been carried out both of the resistance of certain marine soils to the opening of the valves and of the methods used to supplement the ligament.

The figures obtained for the opening of the valves of *Mya arenaria* to a gape of 10 degrees by the application of a steady force may be noted: in clean sand 45 g. mm/mm.^{2}, in muddy sand 30 g. mm./mm.^{2}, and in offshore mud 9 g. mm./mm.^{2}. Since the mean opening moment of the ligament of this species at zero gape of the valves is 3·4 g. mm./mm.^{2}, diminishing to about 1·5 g. mm./mm.^{2} at 10 degrees gape, the action of the ligament would have to be greatly assisted in all of these instances. When the valves are loaded experimentally opening has been observed with loads of up to 26 g. mm./mm.^{2}. It may be noted that this is of the same order as the resistance to the opening of the valves in muddy sand, but that the figure obtained for clean sand is in excess of this.

It can be observed from Fig. 11 that the opening of the valves under load was only of a very short duration. In life the animal does not face problems quite the same as those imposed experimentally. In opening the valves under natural conditions the resistance does not necessarily persist after the valves have opened, whereas experimentally the load remains. Thus the opening peaks obtained by artificial loading would give rise to a sustained gape in natural conditions, and it is probable that activity similar to that shown by the closing pattern would then occur.

In the experiments carried out to determine the resistance of various soils to the opening of the valves a steady force was applied. Agitation greatly reduced the resistance of the thixotropic soils by liquefaction (Fig. 5), and the rapid repeated application of an opening moment to the valves might have a somewhat similar effect. The interval between the peaks in the opening pattern (Fig. 8) is probably too great for this phenomenon to occur, for the soil would re-set between the peaks. Although liquefaction of the soil does not appear to be brought about by agitation, local increase of the water content around the lamellibranch may result from the squirting out of water from the mantle cavity through the pedal aperture.

Quayle (1949) records that in *Venerupis pullastra* a powerful jet of water is directed in front of the animal when burrowing. Other authors, e.g. Jordan (1915) for *Mactra*, and Fraenkel (1927) for the Solenidae, interpret a similar current as serving to loosen the sand in front of the animal. Any such liquefaction of the soil would obviously assist penetration and the opening of the valves. With this means of locally reducing the resistance of the soil the thrust available should be adequate to open the valves of *Mya arenaria* in muddy soils and probably also in clean sand.

The methods by which lamellibranchs open their valves may be conveniently grouped under the following headings: (*a*) the opening moment of the ligament; (*b*) the protrusion of the foot; (*c*) the distension of the fused ventral mantle edges; (*d*) the water pressure in the mantle cavity.

The opening moment of the ligament is the most widespread method of opening the valves. It is of importance throughout the Lamellibranchia except when the ligament is reduced and sometimes absent as in the Adesmacea. The foot is clearly related to the opening of the valves in certain burrowing lamellibranchs, particularly those in which the ventral mantle edges are not fused. The foot of *Anodonta cygnea* has been observed to be protruded, forcing the shell open, when the valves of this species were loaded, as in *Mya arenaria*, in excess of the opening moment of the ligament. Fraenkel (1927) has shown that the blood pressure assists pedal movements in *Solen*, but that it plays only a minor role in comparison to the musculature in inducing changes in the form of the foot. The importance of these factors in the opening of the valves cannot be assessed without further investigations. It is similarly difficult to ascertain the exact role of the distension of the mantle edges. Yonge (1951) suggests that this may be a prime factor in forcing the shell valves apart in deep burrowers, and that the mantle edges may contribute to the opening of the valves in *Platyodon cancellatus*. The thickening of the ventral mantle edge, most marked in *Panope generosa*, the deepest of all burrowers (Yonge, 1951), may also be noted. Further investigations are required to ascertain whether there are any fluctuations of water pressure in the mantle cavity of other deeply burrowing lamellibranchs. It is of interest that Hunter (1949) recorded the shell valves of the rock boring *Hiatella gallicana* being forced apart by water pressure inside the mantle cavity.

While it has been shown that the hydrostatic pressure in the mantle cavity is adequate to open the valves in most soils, only tentative suggestions have been made as to the means whereby this pressure is developed, and further work is required before this may be elucidated.

In *Mya arenaria* the opening moment of the ligament has been shown to be a small factor in the gaping of the valves when buried. Its possible action in continually pressing the valves open against the soil, when the shell is gaping normally, should not be overlooked. The main function of this ligament, and that of other members of the Myacea, is to act as a fulcrum about which the valves may open and about which rocking may take place. Yonge (1951) has observed that the rocking motion of the valves is used in *Platyodon cancellatus*, a member of the Myacea, with a ligament similar to that of *Mya arenaria*, as a means of boring into soft rock and that similar movements occur in *M. arenaria* and *M. truncata* when burrowing. It should be noted that in the unrelated deep burrower *Lutraria lutraria* the arrangement of the ligament is somewhat similar, so that some rocking of the valves may occur. Divarication of the valves at their posterior ends may be related to the presence of the large siphons of these species and their withdrawal into the shell.

SUMMARY

The ligament of *Mya arenaria* allows the valves to open normally and permits a rocking movement of the valves about a dorso-ventral axis to take place. This movement, which makes burrowing possible, is also associated with the divarication of the posterior end of the shell to allow the withdrawal of the siphon.

A method of determining the resistance of various marine soils, ranging from clean sand to offshore mud, to the opening of the valves of *M. arenaria* is described. Even in the offshore mud, which is much less resistant than the sand, the opening moment of the ligament is approximately 6 times too small to cause the valves to gape.

Records have been made of the opening of the valves of *M. arenaria* when loaded experimentally to varying degrees. A closing pattern of activity was observed with loads applied not exceeding the opening moment of the ligament, and with greater loads an opening pattern. The opening of the valves, in the latter instance, is found to be associated with a sudden increase in the water pressure in the mantle cavity. The increase in pressure is shown to be of the correct order of magnitude to open the valves of *Mya* in muddy sand by the application of a steady force. The liquefaction of the sand surrounding the lamellibranch, possibly by the expulsion of water through the pedal opening, would greatly facilitate the opening of the valves.

The author is indebted to Prof. P. G. 'Espinasse for facilities and encouragement and Prof. C. M. Yonge, F.R.S., for much helpful criticism.

REFERENCES

Boswell, P. G. H. (1948). The thixotropy of certain sedimentary rocks. *Sci. Progr.* **36**, 412–22.

Chapman, G. (1949). The thixotropy and dilatancy of a marine soil. *J. Mar. Biol. Ass. U.K.* **28**, 123–40.

Chapman, G. & Newell, G. E. (1947). The role of the body fluid in relation to movement in soft bodied invertebrates. *Proc. Roy. Soc.* B, **134**, 431–55.

Davies, D. M. (1952). Hysteresis in rubber. *Nature, Lond.*, **170**, 937.

Freundlich, H. & Roder, H. L. (1938). Dilatancy and its relation to thixotropy. *Trans. Faraday Soc.* **34**, 308–16.

Fraenkel, G. V. (1927). Die Grabbewegung der Soleniden. *Z. vergl. Physiol.* **6**, 167–220.

Hunter, W. R. (1949). The structure and behaviour of '*Hiatella gallicana*' and '*H. arctica*' with special reference to the boring habit. *Proc. Roy. Soc. Edinb.* B, **63**, 271–89.

Jordan, H. (1915). Über die Art, wie *Mactra inflata* sich in sand einwühlt. *Zool. Jb.*, (Abt. Zool. Physiol.) **35**, 289–300.

Nelson, T. C. (1921). Report of the Department of Biology of the New Jersey Agricultural College Experimental Station. For the year ending 30th June 1920.

Quayle, D. B. (1949). Movements in *Venerupis* (*Paphia*) *pullastra*. *Proc. malac. Soc. Lond.* **28**, 31–7.

Trueman, E. R. (1953). Observations on certain mechanical properties of the ligament of *Pecten*. *J. Exp. Biol.* **30**, 453–67.

Watkins, E. E. (1942). The macrofauna of the intertidal sand of Kames Bay, Millport, Buteshire. *Trans. Roy. Soc. Edinb.* **60**, 543–61.

Yonge, C. M. (1949). On the structure and adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. *Phil. Trans.* B, **234**, 29–76.

Yonge, C. M. (1951). Structure and adaptations for rock boring in *Platyodon cancellatus*. *Univ. Calif. Publ. Zool.* **55**, 401–7.